

# A COMPARATIVE ANALYSIS OF EVOLUTIONARY CHANGES IN ISLAND BIRDS

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## Abstract

The evolutionary pressures of islands are often considered to be quite different to those found in continental systems. The insular flora and fauna and their characteristics that have resulted from this unique suite of pressures have puzzled scientists for centuries. This thesis uses the comparative approach to examine how island passerine birds differ from continental birds. Birds on islands like New Zealand have had an evolutionary history free from mammalian predators, in contrast to continental European species that co-evolved with mammals. Given this difference I examined how island birds differed from continental birds in three ways: sound, smell and appearance. I first looked at differences in the begging vocalisations of New Zealand nestling birds and compared these to the vocalisations of nestling introduced European birds. I expected that introduced species should produce less conspicuous calls given their co-evolutionary history with mammalian predators, while New Zealand birds should have comparatively more conspicuous begging calls. In fact, the calls of the two groups of birds were relatively similar. I then looked at the differences in the volatility (“smell”) of preen waxes between native New Zealand species and introduced European species. I tested the prediction that New Zealand birds, which did not evolve with predatory mammals that located prey by smell, should produce preen waxes that do not function as ‘olfactory crypsis’ as found in continental birds. As found previously, introduced species adopt an ‘olfactory crypsis’ regime in by producing less volatile waxes during incubation. In contrast, most native species showed no shift in wax volatility, with one species even becoming more volatile in the breeding season, supporting a role of predation risk in the evolution of bird odours. Finally, I conducted a survey of evolutionary changes in appearance between insular and continental birds across a variety of isolated island groups, I compared changes in size, mass, wing length, bill size, carotenoid pigmentation, melanin pigmentation and ‘dullness’. I found that island species were significantly larger, with larger bills than their continental counterparts. I also found that carotenoids decreased, and melanin pigmentation significantly increased, resulting in ‘duller’ island species. The reasons for these changes are not clear but

highlight how island environments must differ in selective pressures from that encountered by birds on the continents. Overall my findings confirm that island species differ from continental species, and this extends to both their appearance, and somewhat surprisingly, their smell. My work raises a number of additional avenues for research, including a need to investigate the causes of changes exhibited by island birds.



# Chapter 1

## 1.1 General introduction

### *Background*

The flora and fauna of islands have intrigued scientists for centuries. It was, in fact, the unique fauna of the Galapagos Islands that sparked the curiosity of Charles Darwin, resulting in the formulation of arguably the most important theory of modern science, natural selection. The differences between insular and continental species are still a topic of intense interest by evolutionary biologists, and part of my thesis involves illuminating some of these patterns.

Island species around the world often show similar features. For example, the tameness of the birds Darwin observed on the Galapagos can be found in other islands too. The similarities among insular species are assumed to be a product of the similar pressures exerted by island ecosystems. One such pressure is a lowered risk of predation on island ecosystems because of the lower diversity of predators, especially the absence of mammalian predators, which do not disperse readily to isolated islands. Nest predation specifically is thought to have a strong influence on the life history traits of avian species (Slagsvold 1982; Martin 1995). These life history traits include clutch size, degree of iteroparity, but also behaviours and adaptations of the nestlings themselves that may increase or decrease the probability of nest success. Of course, islands can also differ from continental areas in other respects, such as climate, but it

is the absence of mammalian predators that makes the study of island animals interesting as it provides a model system in which to understand how adaptations towards predators have evolved.

New Zealand, like many islands in the South Pacific has been isolated from the super-continent, Gondwana, for around 80 million years (Cooper and Millener 1993). Strangely, New Zealand remained free of non-volant mammals despite attachment to the Australian continent, on which the evolution of terrestrial mammals was well under way (Rich et al. 1997). This isolation has produced a unique fauna for such a large landmass, with no endemic terrestrial mammals, and a large suite of flightless and distinctive avian species (Cooper and Millener 1993). The lack of mammals has led not only to many avian species adopting the niches of mammals in a continental environment, but the lack of mammalian predation has led to a reduction, and in some cases, complete absence, of predator aversion behaviours (Holdaway 1989; Diamond 1981). These have manifested themselves in repeated evolution of flightlessness, predator naivety and a whole suite of life history traits that lack any sort of crypsis from ground-searching predators like small mammals. New Zealand did however have a number of hawks, falcons and owls, which would have preyed upon small passerines (Holdaway 1989). There are also more opportunistic avian predators, such as cuckoos (*Chrysococcyx lucidus*), and weka (*Gallirallus australis*), which would have had some impact on nest success. This island environment is in stark contrast to that experienced by birds in continental areas. Here a broad suite of small mammalian predators evolved alongside birds for millions of years, leading to adaptations against predation. Predatory mammals rely more heavily on sound and smell to seek out their prey, utilising their highly developed olfactory and auditory senses. Compare this to the bird predators of New Zealand and many other islands,

which are more reliant on sight to locate their prey. The avian species of these two environments have therefore had an evolutionary past with vastly different predation pressures shaping their life history and behaviour and this is expected to lead to differences in the behaviour, appearance, and even smell of birds in the two different environments.

Upon the arrival of humans, and the subsequent introduction of mammalian predators, around 50% of New Zealand's original avifauna has gone extinct (Holdaway 1989). The causes of extinction range from deforestation and habitat loss, to competition with introduced birds and undoubtedly predation from the introduced kioie (*Rattus exulans*), ship rats (*Rattus rattus*), stoats (*Mustela erminea*), weasels (*M. nivalis*), ferrets (*M. furo*), possums (*Trichosurus vulpecula*), and cats (*Felis catus*). The fragility of New Zealand's native species is exemplified by these extinctions, and underscores the need to determine the specific aspects of insular birds that may contribute to their vulnerability from introduced predators.

Duncan and Blackburn (2004) attribute the susceptibility of avian species on isolated islands to three main factors. The first of these factors is that species colonising islands experience evolutionary changes that result in a reduced population size as their distribution becomes more restricted (Wilson 1961). Secondly, the lack of top predators on islands leads to repeated reduction in the ability to fly, and other predator escape responses, drastically increasing their susceptibility to predation upon the arrival of mammals (Diamond 1981). Finally, species found on islands are often characterised by life history traits such as large body mass and low reproductive rates, features that make them more vulnerable to the effects of hunting and habitat loss (Diamond 1989; Holdaway 1989). These patterns certainly apply to New Zealand, but is their incidence unique, considering the size of the New Zealand landmass, or do

island birds in general share features that make them different, and perhaps more vulnerable, to introduced mammalian predators? In this thesis I explore the differences between island and continental birds, by examining 3 traits that I thought might change in birds isolated on islands with few predators: these can be broadly categorised as “sound”, “smell” and “appearance”, and how I examined these traits are outlined below.

### ***Outline of thesis***

Using New Zealand as a case study for island ecosystems, the first two data chapters of my thesis look at the effects of differences in predation pressure on the evolution of two life history traits of birds; the sound of begging nestlings and the smell of the adults. The third data chapter then takes a broader view by examining changes in colour and morphology of birds on a whole array of isolated islands. All three data chapters are based on the premise that the life history traits, appearance, morphology and behaviour of a species, are a product of evolutionary adaptations to its environment, whether an island or a continent, and the species that they share that environment with.

The first trait I will focus on is the begging of nestling birds. This is a signal between parents and their young, which in birds takes the form of colour changes of the mouth (Kilner 1997), posturing and vocalisations (Kilner 2002). For my thesis, however, I focus just on the vocalisations. The conspicuous nature of these vocalisations has caught the attention of scientists, as they appear to be costly and unusual behaviours. The intensity of begging is predominantly driven by hunger level (Mondloch 1995), but as with any signal, this is vulnerable to exploitation by

dishonest users, such as a potential predator. Nestlings may beg more intensely in an attempt to sequester more food from their parents than they can energetically afford to provide, along with trying to compete with nestmates. This is further confounded with the decreased relatedness of nestmates (Birschie et al. 1994), which can further increase the strength of the begging signal.

Whatever benefit begging provides in the form of more parental care, the behaviour also has its costs. The costs of begging include both a metabolic cost (Chappell and Bachman 2002), and the cost of attracting nearby predators (Halupka 1998). The metabolic cost is not thought to be very high from recent studies, so predation appears to be the most likely factor constraining the vocalisations of nestlings. This manifests itself in quieter, shorter and higher frequency calls of species that are under greater predation pressure (Briskie et al. 1999). In contrast to this, species under very low predation have louder, longer and lower frequency calls. In chapter 2, I test the hypothesis that the evolutionary history of New Zealand's native birds has shaped their nestlings' vocalisations in such a way that they are more conspicuous than those of European introduced species, which co-evolved with mammalian predators in their native range. I use the comparative method to test if differences between the two groups are due to their differing evolutionary history with predation risk.

The next chapter (chapter 3) focuses on the uropygial secretions or preen waxes of native New Zealand passerines and compares them to the preen waxes of introduced European passerines. Preen waxes are oily secretions of the uropygial gland, a bird's sole sebaceous gland found just dorsal to the base of the tail (Elder 1954). Birds use their bill to anoint their feathers with these waxes during bouts of preening, giving the gland its common name of the preen gland. The function of

these waxes is still argued, but a summary of their functions includes: water repellency, UV protection, ectoparasite defence, microbial defence, and a reduction in feather wear and tear (Elder 1954; Kolattukudy et al. 1987; Montalti and Salibian 2000; Sinninghe Damste et al. 2000; Sweeney et al. 2004). The waxes are made up primarily of long-chain esters, fatty acids and alcohols that vary in their attachment and orientation to create a vast and varied range of waxes (Sinninghe Damste et al. 2000; Reneerkens et al. 2006). These different waxes have different levels of volatility, thus differences in wax composition between species, or within a species due to season or sex, will result in different birds having different “smells”.

Much of my work has been stimulated by the research of Reneerkens et al. (2002) on the lipid profiles of sandpipers (family: Scolopacidae), a group of migratory shorebirds found in the northern hemisphere. The work initially discovered that the makeup of these waxes varied on temporal scales that coincided with the breeding season. Further investigation found that in the breeding season, greater molecular weight diesters were being produced, whereas in the non-breeding season, lighter molecular weight monoesters were produced. The discovery that this shift was only exhibited by the incubating sex provided an indication that it was directly linked to the incubating phase of the breeding cycle (Reneerkens et al. 2007). Reneerkens et al. (2005) proposed that this shift of producing heavier molecular weight secretions in the breeding season was an attempt to increase olfactory crypsis. The theory was proposed that mammalian predators using olfactory cues to locate prey would find individuals that used less volatile secretions harder to find. In my thesis I analyse the preen waxes of a range of native and introduced passerines in New Zealand in both the breeding and non-breeding seasons to test this “olfactory crypsis” hypothesis. As the introduced continental passerines have evolved alongside numerous mammalian

predators, they would be expected to exhibit adaptations, like olfactory crypsis, to enhance survival in the presence of such predators. If introduced continental species show a shift to less volatile waxes in the breeding season, but the native species, which evolved without mammalian predation, do not, then this would help to support the olfactory crypsis hypothesis.

In chapter 4, I turn my attention more globally and conduct a pairwise comparative study of island and continental birds across a large variety of island groups, with the objective of looking for general patterns in how island birds differ in size, colour and bill morphology from their continental relatives. Amongst the earliest observations of insular species was that they were inherently larger than their continental counterparts (van Valen 1965; Lomolino 1985). This phenomenon of predictable size change on island has been explained by a number of theories. These range from niche expansion (Scott et al. 2003) to founder effects (Mayr 1942), to changes in interspecific competition and predation (Lomolino 1985). Mayr (1942) also suggested that the reduced population sizes on islands were responsible for the loss of such isolating mechanisms as male ornamentation. This explanation came after observing the loss of many secondary sexual characteristics in island males, such as bright colours, long tails and other ornamental traits (Peterson 1996).

Although these earlier studies have provided some insight into the patterns of morphological change in island birds, they have either suffered from a restricted sample of islands or have lacked rigorous statistical methods of controlling for the potentially confounding effects of phylogeny. My study looks specifically at changes in body size, bill size, carotenoid pigmentation, melanin pigmentation and ‘dullness’ of island species and their continental counterparts. I did this across a wide range of islands and used the pairwise comparative method based on that used by Møller and

Birkhead (1992) to control for phylogenetic effects. I hypothesised that isolation on islands would result in changes in body size and colouration due to the differences in environments between islands and continents, including those due to differences in the levels of predation risk.

Finally, in the last chapter (chapter 5) I provide a general discussion of my findings. Island avifaunas have puzzled scientists and explorers since Peter Mundy first encountered the dodo on the remote island of Mauritius in the 1600s (Diamond 1981). Subsequent discoveries of flightless, and other peculiar avian species on isolated islands have continued to fuel this interest and lead to the formulation of much evolutionary theory. Recognition of how animals differ between islands and continents may allow a better understanding of the evolution of island birds and why so many are vulnerable to exotic mammalian predators.



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## **Chapter 2**

# **A comparative study of the nestling vocalisations of New Zealand and introduced European passerines.**

### **2.1 Abstract**

The signalling of need through begging is a phenomenon exhibited by offspring attempting to solicit feedings from their parents. In birds this takes the form of posturing, colour changes of the mouth, and vocalisations. Apart from functioning in obtaining food, these conspicuous behaviours are under selective pressures from predation risk, as begging can also attract the attention of a predator. I performed a comparative study on the begging vocalisations of native New Zealand and introduced European passerine nestlings. New Zealand species evolved in the absence of predatory mammals and as a consequence might be expected to produce vocalisations that are louder, with a lower frequency and broader frequency range, and therefore be more conspicuous than European birds, which co-evolved with mammals. Although limited by a small sample of species, I did not find support for this hypothesis and instead found that the begging vocalisations of New Zealand and introduced European nestlings were similar. Native New Zealand birds had calls with a higher frequency, but this was not quite significant and opposite to that predicted. However, the frequency saturation of calls appeared to differ slightly between the two groups with those of introduced species being characterised by chevrons, stacked in harmonic groups, while the calls of most native species had broader, saturated bandwidths, and utilised all frequencies in their range. Despite a variety of confounding factors, my preliminary survey of begging call structure suggests the signals of native birds do differ from European species although this needs to be confirmed with a larger sample of species and why it should differ needs to be studied further.

## 2.2 Introduction

The young of many animals attempt to convey their hunger level to their parents (and thus solicit parental care) through a variety of behaviours known as begging. This behaviour is almost ubiquitous among avian species, with the signals ranging from changes in mouth colour (Kilner 1997), to vocalisations and posturing displays (Kilner 2002). Typically, the hungrier a nestling is, the more often and more intensely it will beg (Mondloch 1995). Young animals, however, face a trade-off between producing signals that effectively convey hunger levels and signals that at the same time will not attract predators to the nest (Halupka 1998). The end result is a signal moulded by the nestling's requirements for food, and their prerequisite for crypsis from predators.

The locatability of a nestling vocalisation, whether by parents or potential predators, is determined by a number of physical features of the call. The first and simplest of these is the decibel level or loudness of the vocalisation; generally as decibel level increases, the locatability of the sound also increases (Wiley and Richards 1982). A second feature of a call is its frequency. Different frequencies of sounds attenuate through the environment differently, with higher frequency sounds attenuating faster as they are absorbed more readily by vegetation and atmospheric perturbations. In contrast, lower frequency sounds travel further and are affected less by scattering on vegetation and atmospheric absorption; thus low frequency vocalisations are generally more easily located by a listener than high frequency vocalisations (Wiley and Richards 1982). Finally the bandwidth, or range of frequencies used by an individual will affect how easily the sound can be located. Vocalisations with wide bandwidths that utilise sounds from a large number of

frequencies are easier to locate by mammalian listeners through what is known as phase-difference detection (Briskie et al. 1999). Thus, begging vocalisations that have a wide band width, low frequency, and high amplitude will be most readily locatable by a listener, whether a parent or a predator.

As any vocalisation has the potential to be heard, not only by the intended receiver but also by a potential predator, selection should favour calls that maximise the efficiency of communicating with the receiver but minimise the risk of the alerting a predator to the location of the broadcaster. This means that species evolving with a high risk of predation should evolve calls of lower decibel level, and narrow, yet high bandwidths. Species that face a low risk of predation however, should evolve vocalisations with broad bandwidths, high decibel levels and lower dominant frequencies. For example, the birds of most continental areas have evolved with intense mammalian predation and this has resulted in adaptations that reduce the possibility of predation of their nestlings by unintended listeners like rats, and mustelids. In contrast, the birds of New Zealand had an evolutionary history free from mammalian predators, suffering predation only from other avian species (Holdaway 1989). With the differing suites of predators come differing sets of predatory cues by which the predators locate their prey. Mammals primarily use sound and smell, whereas raptors and other opportunistic predatory birds (cuckoos, *Chrysococcyx lucidus*, weka, *Gallirallus australis*) predominantly utilise sight to find prey. This suggests that the begging vocalisations of nestling birds should differ between continental areas where birds co-evolved with mammalian predators, and islands like New Zealand, where the birds evolved in the absence of mammals.

In a study of nestling vocalisations of birds nesting in an Arizona woodland, Briskie et al. (1999) confirmed that the structure of the begging calls among species



was correlated with differing risks of nest predation. Those species subject to higher rates of predation generally had quieter calls (low amplitude) and calls of a higher pitch (greater frequency). Forsman and Mönkkönen (2001) also found some evidence for the role of vocalisation locatability in the evolution of mobbing calls of adult birds. Mobbing calls are used to attract conspecifics and other species to “gang up” on potential predators. They found that the acoustic properties of the mobbing calls converged on signals that were of short duration, repeated, and had broad bandwidth pulses of sound. They suggested that these properties made the calls more easily locatable and thereby increased the recruitment of nearby individuals.

Although more locatable calls might be advantageous in environments with low predation risk, as they function to increase parental attention, such calls are not cost free and there are likely to be other limits that constrain a continued exaggeration or increase in begging call conspicuousness. For example, much work has gone into quantifying the metabolic cost of begging, with the idea that increased begging costs more energy. Although the results have been mixed, the consensus so far is that costs are present but minimal, and have little effect on an individual’s fitness (Chappell and Bachman 2002). The energetic costs of begging, though still hotly debated, may provide one of the only forces curtailing the evolution of increasingly more extravagant begging displays in nestlings free from predation costs.

Apart from the physics of sound transmission, begging vocalisations are also influenced by a number of other variables, which have an effect on both the intensity and audible characteristics of the calls. For example, brood size can affect vocalisations to two main ways. Firstly, decibel level is a cumulative measure, so more individuals make more noise, increasing the ‘loudness’ of a given nest. Secondly, increased brood size can increase competition, resulting in nest mates

increasing their begging intensity in order to sequester more feedings from their parents (Roulin 2002). Competition levels among nestlings is further confounded by the relatedness of the siblings; nestlings that are only half-sibling due to extra-pair paternity should be more selfish and therefore increase their begging intensity to compete against less related nest mates (Briskie et al. 1994). Finally, nesting site can also have a strong effect on the vocalisation of the nestlings. Cavity nesters are notoriously loud compared to cup nesting species, possibly due to relaxation of predation pressure because of safer, harder to locate nests (Redondo and Arias de Reyna 1988). Thus, a number of factors are known to affect the structure of begging calls, although it is still expected that differences in predation risk should play a role in shaping the exact nature of begging behaviour. In other words, for a given brood size, species that evolve with higher risk of predation should produce begging calls that are less easily locatable than species with the same brood size but that evolved with a low risk of predation.

In this study I use the differing evolutionary history of two groups of birds; introduced continental species, and native New Zealand species, to compare the characteristics of their vocalisations. Native New Zealand birds with their history free of mammalian predators are predicted to be louder, have broader bandwidths and have lower overall and maximum frequencies. Introduced species that have evolved with the predation risk of mammals are predicted to produce vocalisations that are quieter, with narrower bandwidths and have higher overall and maximum frequencies. Confirmation of such predictions could have important implications for understanding both the evolution of vocal signalling in birds but also one potential mechanism behind the extinctions of large numbers of avian species on island systems where birds were not exposed to mammals until introduced by humans.

## 2.3 Methods

### *Study site and species used*

Begging vocalisations were recorded from a variety of introduced and native New Zealand passerine nestlings. All the passerine species I studied produce altricial, helpless young, which shortly after hatching, begin to beg for food. The signals produced by these nestlings are a complex combination of posturing and vocalisations, which are thought not only to increase feeding rate by increasing nest visitation by parents, but also to increase an individual's allocation of a given bout of feeding (Mondloch 1995). For my study, however, I only examined the structure of the vocalisations.

I made recordings from birds nesting in Kowhai Bush, Kaikoura between 2006-2008. These were also supplemented with recordings from the breeding seasons of 1998 and 1999, also from Kowhai Bush, made by J. Briskie. The bush consisted primarily of kanuka stands and intermediate native podocarp forest. Native birds analysed included the bellbird (*Anthornis melanura*), brown creeper (*Mohoua novaeseelandiae*), fantail (*Rhipidura fuliginosa*), grey warbler (*Gerygone igata*), South Island robin, (*Petroica a. australis*), and rifleman (*Acanthisitta chloris*). The introduced species recorded included Blackbird (*Turdus merula*), dunnock (*Prunella modularis*), redpoll (*Carduelis flammea*), song thrush (*T. philomelos*), and starling (*Sturnus vulgaris*). The silvereye (*Zosterops lateralis*) was grouped with the introduced species despite its recognition as a native species as it has only colonised New Zealand in the last ~150 years from populations in Tasmania, where they

evolved with a variety of native mammalian predators (thus they share a common evolutionary history with the introduced European birds).

All species were open-cup nesters with the exception of the starling and rifleman, which use artificial nest boxes or natural cavities. Note that the grey warbler also builds an enclosed hanging nest, except for a single side opening.

### ***Recording Procedure***

The recording protocol was based on that used in Briskie et al. (1994, 1999). The recordings were made comparable between species by standardising recording level, nestling development and microphone distance. To control for nestling development, nestlings were only recorded within 24 hours of their primaries emerging from the sheaths (pins broken). A Sony TCM-5000EV, and a Sony professional Walkman model WM-D3 were used for recording the calls with a Sony PC-62 stereo microphone. Calls from two different tape recorders were analysed but these were calibrated by the recording of a standardised tone at the beginning of each tape on each machine. The microphone was placed 15 cm above the nestlings, except in the case of the rifleman, whose nest box restricted placement to between 10 and 15 cm. This microphone was then attached to a 50 m cable, which enabled me to retreat fully out of sight. The parent would readily resume feedings within a few minutes of setting the microphone. Only begging bouts in response to parental visitation was recorded. This was done by listening for the approach of the parents to the nest, and then manually initiating the recording upon the arrival of the parent. At least one nestling was weighed as an estimate of chick mass. Between 1 and 7 nests (mean 2.8 nests) were recorded for each species.

### *Analysis of Recordings*

The recordings were converted into sonograms and analysed using Raven 1.2 for Mac OS X, from Cornell University. The procedure for the analysis of the sonograms was based on that used by Briskie et al. (1999). For each begging bout I analysed the first 1 second from the time the parents arrived. The following variables were then calculated from this 1-sec sample: frequency at maximum amplitude (Hz), and maximum amplitude (dB). Next, four discrete calls were taken from each bout, other than those occurring in the first second, from which the following variables were calculated: maximum and minimum frequency (Hz), frequency range (Hz), frequency saturation, and length (s) (see figure 2.1). This process was carried out on three to four bouts from each nest, with the resultant data pooled and means for each nest calculated.

The mass of the nestlings was controlled in all analyses, as birds of different sizes are able to produce different ranges of frequencies and amplitudes. This is due to a combination of the effects of differences between species in syringeal mass (thickness of the membranes of the syrinx), and the size of the bill, trachea and buccal cavity, all of which influence the tone and frequency of sounds (Ryan and Brenowitz 1985; Wallschläger 1980). As loudness is additive, nests with more chicks will be louder, I therefore also controlled for number of nestlings in my analyses. Finally, I controlled for potential phylogenetic effects by using the Comparative Analysis by Independent Contrasts software by Purvis and Rambaut (1994). This analysis uses contrasts between taxa to control the confounding effect of birds sharing similar begging call characteristics because they share a common ancestor, rather than as a consequence of different levels of predation in their evolutionary history.

### ***Statistical Analysis***

Firstly, a regression was performed on each of the variables I measured, against body size to control for the confounding effect of mass. This was only significant for the 'max power' ( $F = 5.12$ ,  $p = 0.047$ ). None of the other variables were significantly correlated to body size, so the analysis was performed on the raw data for those characteristics. For the max power, the residuals of the regression analysis were used in place of raw observations. As not all data were normally distributed, I used a Mann-Whitney test to examine any differences between the two groups of birds with a critical value of  $p = 0.05$ .

## **2.4 Results**

There was no significant difference in either frequency saturation or frequency range (figure 2.2) between introduced birds from Europe, which co-evolved with mammalian predators, and native New Zealand birds, which did not evolve with mammals ( $W = 48$ ,  $p = 0.17$ ;  $W = 49$ ,  $p = 0.13$ , respectively). Maximum frequency showed the greatest difference (figure 2.3), with native New Zealand birds having calls with a higher frequency, but was not quite significant and in the direction opposite of that predicted ( $W = 51$ ,  $p = 0.066$ ). Call length ( $W = 38$ ,  $p = 0.94$ ) and residuals of maximum power ( $W = 40$ ,  $p = 0.94$ ) also showed no significant difference between native and introduced species of birds. None of these results changed when I controlled for phylogeny (all  $p > 0.05$ ).

Although I found only small differences between European and native New Zealand birds, the measures I used to characterise vocalisations omit some important differences between the structures of the calls that are not easily quantified (figure 2.4). For example, the calls of the introduced species, with the exception of the cavity-nesting starling, are made up of chevrons, stacked in harmonic groups. In contrast, the calls of most native species have calls with broader, saturated bandwidths, and utilise all frequencies in their range, unlike the introduced species (see fig 2.4). I attempted to quantify this using the measure ‘frequency saturation’ but this was confounded by differences between open and cavity nesting birds. However, when I omitted cavity nesters from the analysis, the difference between native and introduced species was insignificant for frequency saturation ( $W = 36$ ,  $p = 0.095$ ). Frequency range was similarly confounded by cavity nesters, but when omitting them from the analysis, frequency range was still insignificant ( $W = 36$ ,  $p = 0.095$ ).

## 2.5 Discussion

Despite the expectation that native New Zealand birds and introduced European birds should differ in the structure of their nestling begging vocalisations as a result of their differing evolutionary histories with mammalian predators, I found little evidence that they differed in the ways expected from predictions made from an understanding of how the locatability of sounds varies with their structure. The most significant difference I found was a weak trend for New Zealand birds to have calls with a higher maximum frequency, which contradicts the predation hypothesis that species under lower predation should utilise lower frequencies. I also found a weak trend for New Zealand birds to have calls with a greater frequency range, which

supports the predation hypothesis, in that species under lower predation pressure should utilise broader bandwidths, encompassing more frequencies. The saturation of these frequencies, although also not significant in my analysis was greater in the native species. The rest of the call characteristics I measured were highly non-significant. Nevertheless, my results should be treated with caution and viewed more as a preliminary examination of nestling vocalisations as my sample size of species was small and this made it difficult to control other confounding aspects of life history such as nest site and brood size differences. Given these problems, and the fact that some patterns were apparent, these findings suggest that the topic is worthy of future study.

There were a number of confounding factors potentially affecting my analysis. The first was body mass differences among species. Although previous research found that larger individuals produce louder sounds, this did not seem to be the case in my study. This is possibly due to differences in numbers of nestlings between species, which has a cumulative effect on decibel level, or the effects of including two cavity nesting species in my analysis, which produce unusually loud vocalisations. Analyses were performed without the cavity nesters but the results remained insignificant, suggesting that nest site alone is not confounding my results. The intensity of begging, including the loudness, call length and temporal frequency is also known to be affected by hunger levels (Sacchi et al. 2001). The hunger levels of a given nest could vary depending on time of day, time of year, food availability and parenting ability. Such variables were impossible to control in my study, and I assumed that hunger level varied randomly with respect to the other variables and that it was unlikely to differ systematically between native New Zealand birds and introduced species, although this assumption needs to be tested.



Another factor I was unable to control in this study was differences in relatedness of the nestlings among species. Briskie et al. (1994) found that the loudness of a nest increased as a function of the decreased relatedness of the nestlings. The amount of mixed paternity is variable between species with some monogamous species having 0% mixed paternity (e.g. *Miliaria calandra*) while other more promiscuous species having upwards of 50% mixed paternity (e.g. *Emberiza schoeniclus*; Birkhead and Møller 1995). Dunnocks (*Prunella modularis*), which had relatively loud calls in my study, and call with a broad frequency range, are known to be notoriously promiscuous in Europe (Burke et al. 1989). If rates of mixed paternity are as high in the introduced New Zealand dunnocks, then this may explain the exaggerated begging vocalisations of this species. Likewise, European starlings (*Sturnus vulgaris*) are known to be facultatively polygynous, and subject to egg-dumping, extra-pair copulations and rapid mate switching (Smith and von Schanz 1993). The result is broods often containing half-siblings or nestlings that are not siblings at all, due to egg-dumping. Rates of mixed paternity are not known for most species included in my study, although the South Island robin is known to have relatively low mixed paternity (J. Briskie pers. comm.).

The evolution of life-history traits, like begging vocalisations, is most probably the result of a combination of selective pressures, including predation risk, relatedness of nestlings, nest site, and perhaps other factors yet to be identified. Even if differences in predation risk have not shaped the evolution of begging vocalisations in New Zealand species, it is likely that birds here have experienced other differences in evolutionary pressures to that of the birds of the European continent. If predation risk has not part been important in the evolution of begging calls in New Zealand birds, then the vocalisations of native species may give us an insight to the how signals

evolve in this context, and perhaps further our understanding of the origins of parent-offspring conflict.

The birds of New Zealand have been exposed to introduced rodent predators for at least 1000 years, since the introduction of kiore (*Rattus exulans*) (Holdaway 1989). This presents the possibility that New Zealand birds may have already made some adaptations to an environment containing mammalian predators, and as I recorded native birds only on the mainland where native birds have co-existed with introduced mammals for at least the last several centuries, it is possible that the lack of difference I found was due to the fact that the native birds have already changed their begging call structure in response to the novel predation risk. Although mustelids (*Mustella spp.*) (Marshall 1963), and possums (*Trichosurus vulpecular*) (Pracy 1962) have only been here for 100-150 years, the adaptations evolved against rodent predation over the 1000 or so years since their arrival could have a similarly cryptic affect against these predators as well. Interestingly, the traits that are most similar between native and continental species are those that are probably the most plastic, or easiest to change. Loudness (decibel level), and call length were very similar between the two groups of birds, and perhaps represent characteristics that could readily be changed by a plastic response to varying levels of predation risk. Maximum frequency and frequency range, however, would probably be harder to manipulate in the short term, as they are restricted by the morphology of the syrinx (Ryan and Brenowitz, 1985; Wallschläger, 1980). Thus the characteristics that could be more easily changed seem to be the ones that are most similar between the two groups of birds. The characteristics which are determined by morphology, however, are the most different between the two groups, owing possibly to the more involved processes required for their adaptation. The possibility that New Zealand birds may have adapted in part to novel risks of

mammalian predation in the last 300-500 generations may thus provide an explanation for the similarity of some vocalisation traits between New Zealand and introduced European birds. At present, I cannot rule out this hypothesis, but it could be tested by comparing the begging vocalisations of native species that occur both on the mainland (where they have been exposed to exotic predators) and on offshore islands on which exotic predators have never been introduced, such as the Poor Knights or Snares islands.

Our understanding of begging evolution is based on the premise that begging more intensely is advantageous for it results in the individual sequestering more food. What could potentially confound this, especially in instances of species with low predation risk and high relatedness, is that it is not the begging signal alone that establishes food allocation, but the begging signal relative to nestmates. In contrast, it is the begging signal of the nest as a whole that is the cue to which predators use for finding a nest. It would therefore be this total brood signal that is modified by evolutionary adaptations against nest predation. In the case of New Zealand birds, with a history of low nest predation, and potentially very high relatedness, nestlings may not be selected to be as selfish when producing begging signals. This reduced selfishness would manifest itself in a reduced overall begging signal, as individuals are not consistently trying to sequester feedings in competition with their nestmates. Even if relatedness were similar between European and New Zealand birds, other variables such as feeding rate, which is considerably higher in New Zealand birds (J. Briskie, pers. comm.), would provide the context for exhibiting greater selfishness in introduced birds, which in turn would mean more competition over less feedings. Thus, factors such as increased feeding rate, and possibly greater overall relatedness of nestlings may have reduced the need for increased begging intensity in New

Zealand birds despite the lower risk of escalating begging signals due to predation risk.

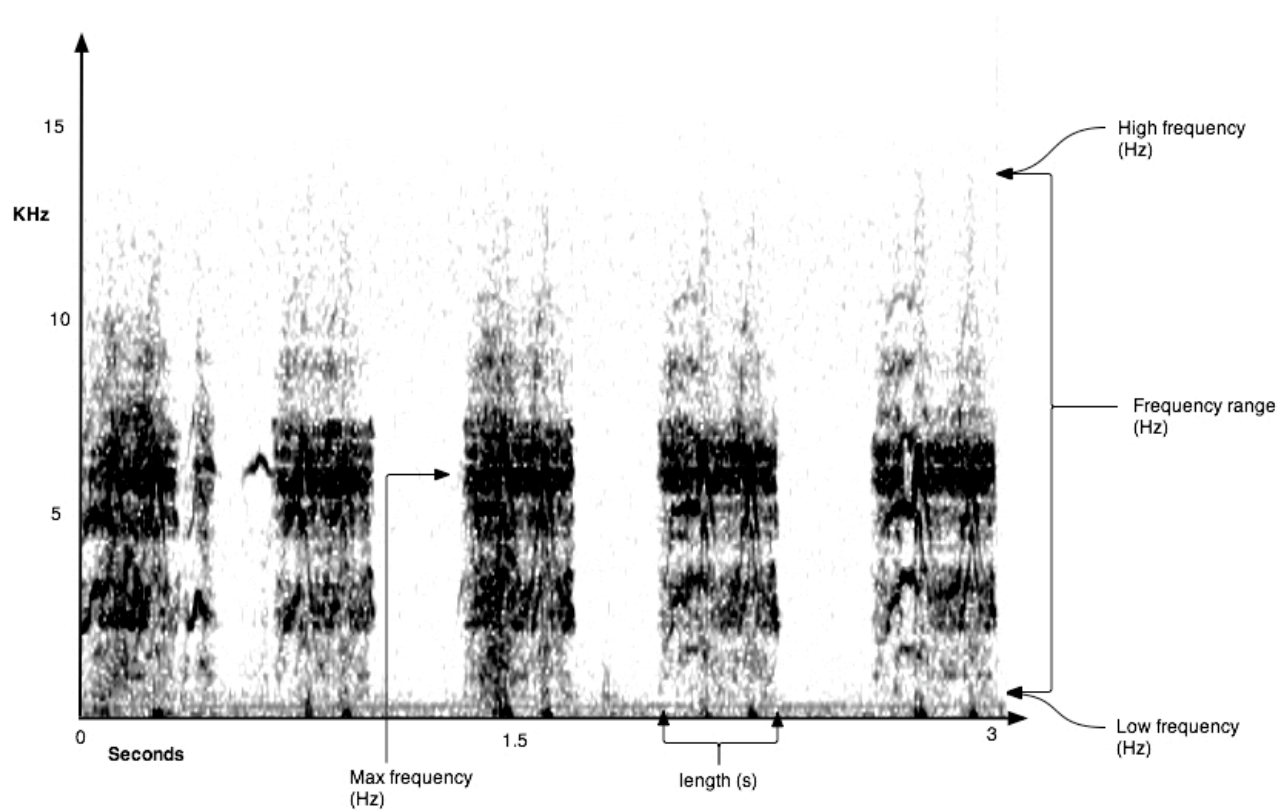
The begging vocalisations of island and continental birds are likely to have been under different selective pressures in their evolutionary past given differences in the environments. The result is the evolution of complex signals, which function in nestlings obtaining the same goal, namely, gaining the most food from their parents as possible. Although my results did not support the predation hypothesis, the unique evolutionary history of New Zealand birds invite further research into how this has affected the behaviour of chicks and their parents at the nest.

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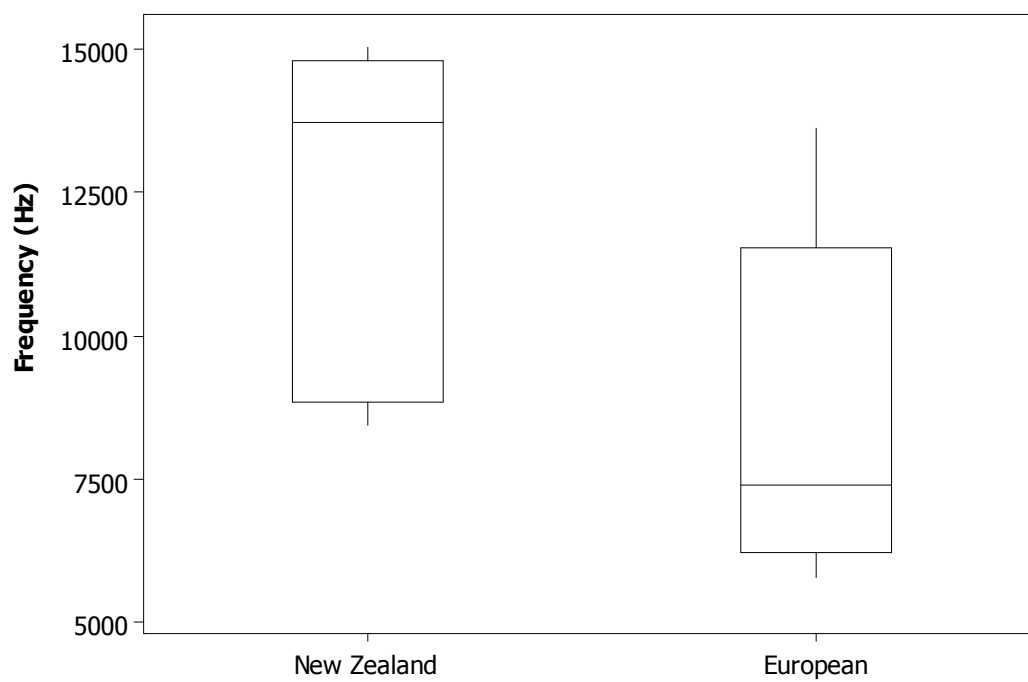
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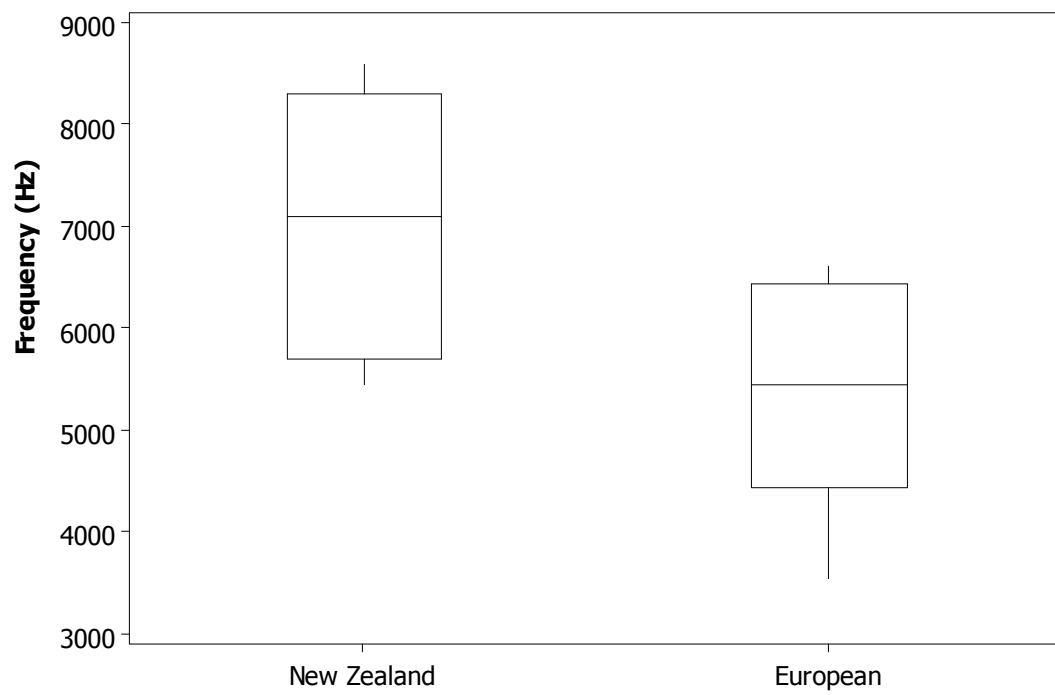


**Figure 2.1.** Figure detailing the measurement of the following characteristics from a sonogram: maximum power, call length, frequency range and high and low frequency.

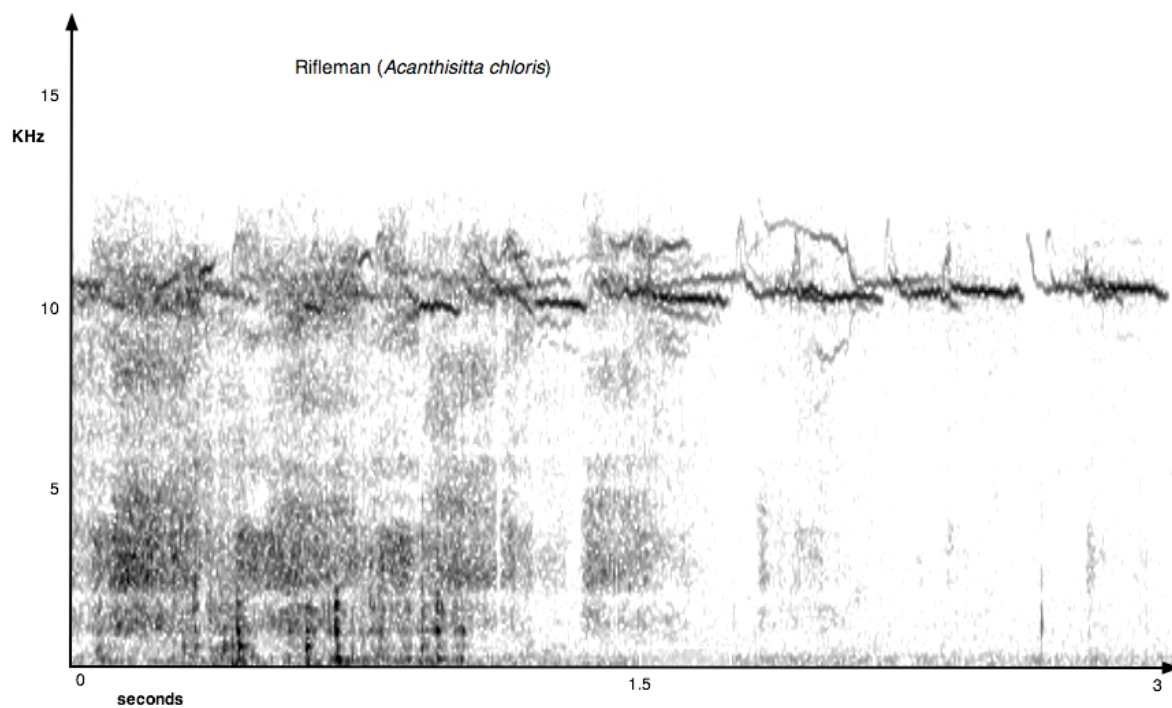
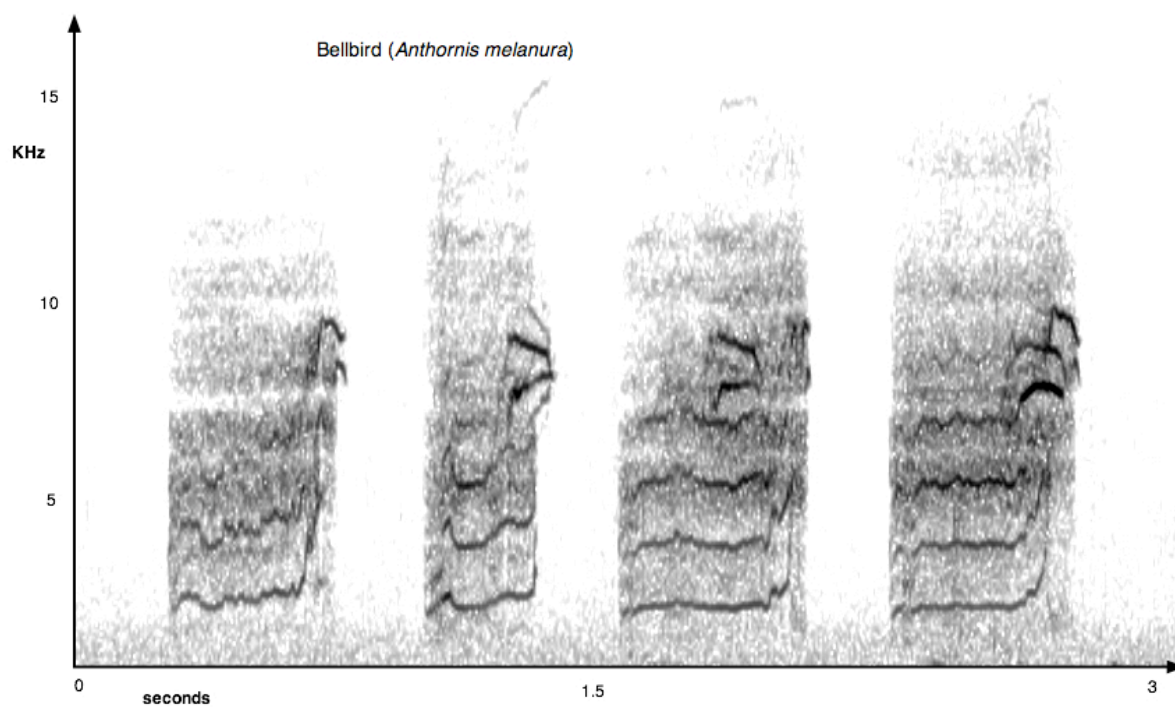




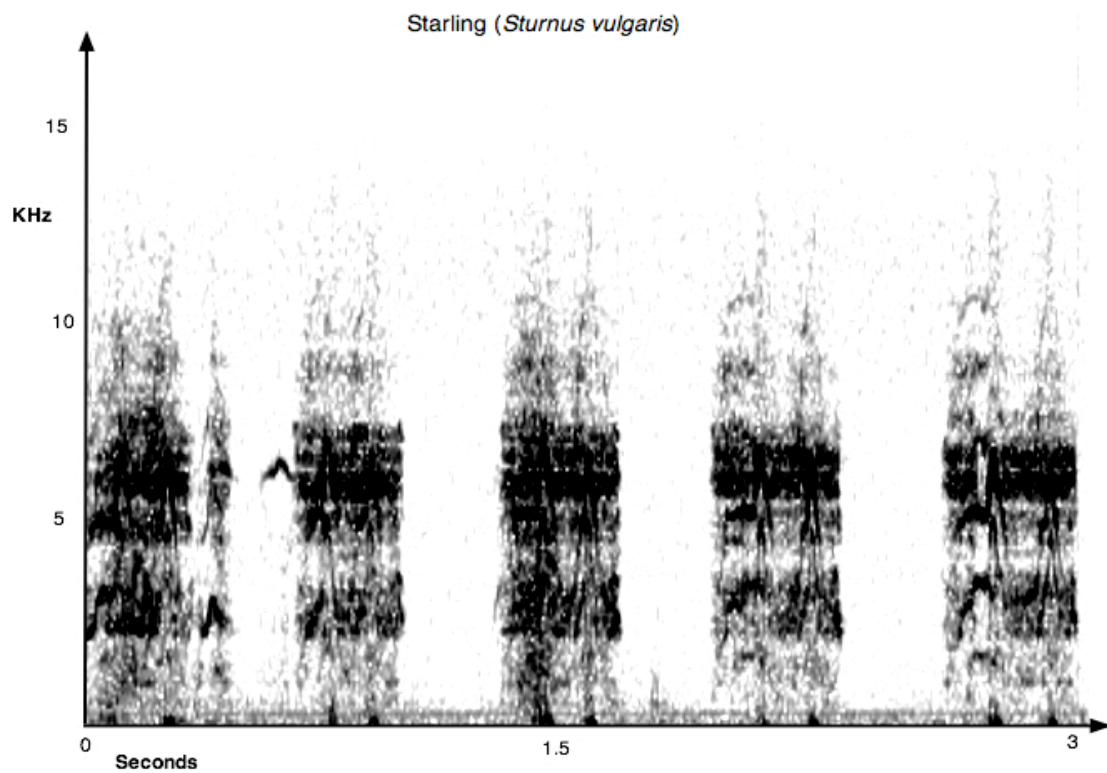
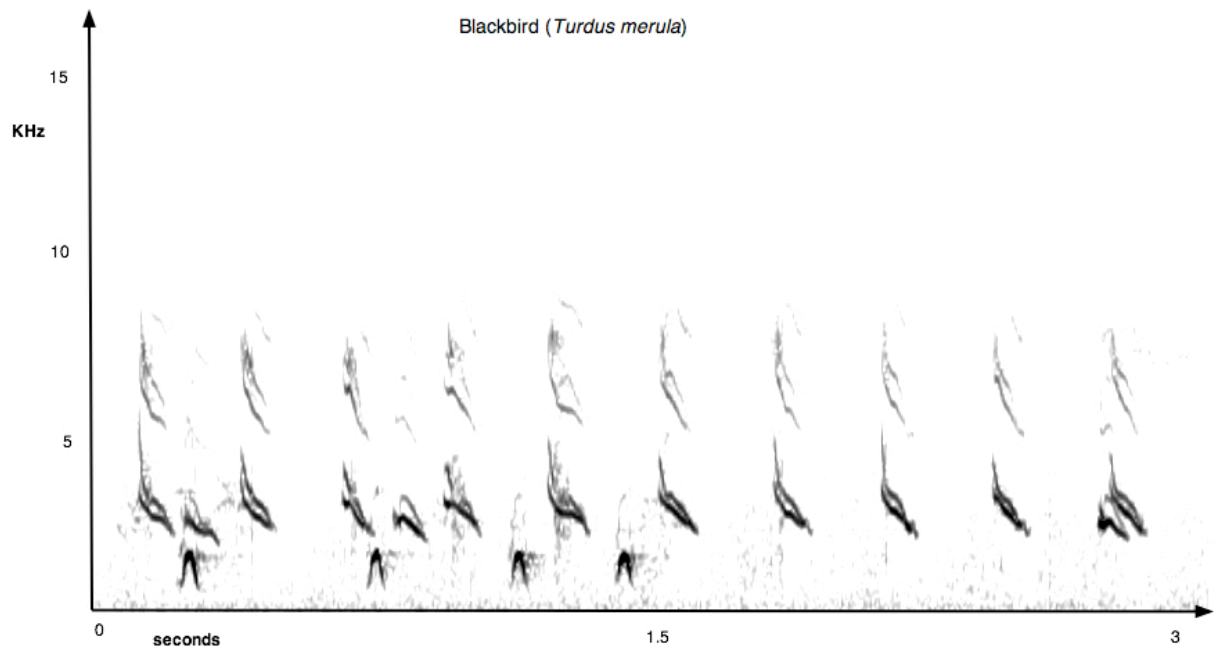
**Figure 2.2.** Box-plots comparing the frequency ranges of nestling begging calls in native New Zealand birds ( $n = 6$ ) and introduced European birds ( $n = 6$ ).



**Figure 2.3.** Boxplots comparing the maximum frequencies, the frequency at which most energy is attributed, of nestling begging calls in native New Zealand birds ( $n = 6$ ) and introduced European birds ( $n = 6$ ).



Continued...



**Figure 2.4.** Examples of sonograms produced from the begging vocalisations of two species of introduced nestling birds (blackbird and starling) and two species native nestling birds (rifleman and bellbird).

## Chapter 3

# Olfactory crypsis: do New Zealand passerines modify their uropygial secretions during the breeding season?

### 3.1 Abstract

The unique avifauna of New Zealand had a long evolutionary history without predation by mammalian predators. The birds of the continental areas however, have co-evolved with mammals, and presumably evolved adaptations against such predators. As many predatory mammals use olfactory cues to locate their prey, one might expect differences between the “smell” of native New Zealand birds and those of continental areas as a result of their differing evolutionary history. In this chapter I consider this ‘predation’ hypothesis when investigating changes in the “smell” or volatility of the uropygial secretions (preen waxes) of New Zealand (native) and continental (introduced) passerines. More specifically I determined if introduced species showed a shift to producing less volatile preen waxes in the breeding season, a possible tactic for increased olfactory crypsis. I then looked for a similar shift in New Zealand species that evolved without mammalian predators and therefore would not require such olfactory crypsis. I found, as predicted, that almost all introduced species produced less volatile preen waxes in the breeding season than in the non-breeding season. In contrast, for all but one native species, no shift to less volatile waxes was observed in the breeding season. The only native New Zealand to shift wax volatility was the South Island robin (*Petroica a. australis*), which changed to more volatile waxes in the breeding season, the opposite to the ‘predation’ hypothesis. These results indicate that olfactory crypsis is a likely reason birds shift to less volatile preen waxes in the breeding season in continental areas but fail to do so in areas without mammalian predators. My results also suggest the possibility that the waxes of some New Zealand birds may have become more volatile and “smellier” once freed from the constraint of maintaining crypsis from mammals that is necessary on continental areas.

### 3.2 Introduction

Birds produce a wide range of volatile chemical compounds, some of which are detectable by human observers (Hagelin and Jones 2007). For example, in a survey of the literature, Weldon and Rappole (1997) found that 80 genera of birds from 10 families and 17 orders were recorded as being odorous and/or unpalatable. These odours ranged from the citrus-like smell of crested auklets (*Aethia cristalella*) to the rancid smell of the hoatzin (*Opisthocomus hoazin*). Even some New Zealand birds are well known by their smells, with the kakapo (*Strigops habroptilus*) likened to the odour of a violin case (Butler 1989) and kiwis (*Apteryx* spp.) giving off a strong, easily identifiable odour (Taborsky 1988).

Bird odours are produced in a variety of ways, ranging from secretions of the skin, to excrement, to environmentally sequestered compounds retained on the body (Weldon and Rappole 1997; Hagelin and Jones 2007). However, for the majority of birds the most likely source of their smell is the waxes and oils produced by the uropygial or preen gland. The uropygial gland (UG) is located dorsal to the levator muscle of the tail in a mass of fatty tissue (Elder 1954). The UG secretes a variable array of lipid-based products through one to five openings via a teat-like structure surrounded by specialised feathers (Elder 1954; Montalti and Salibian 2000). The products of the UG, in most species, are complex combinations of di- and monesters of varying chain lengths. The attachment of fatty acids and alcohols to these chains in varying orientations and positions, results in a secretion containing hundreds of different wax esters (Sinninghe Damste et al. 2000; Reneerkens et al. 2006). The waxes are then anointed to the feathers using the bill during bouts of preening (Elder 1954).

Although still debated, the primary function of the UG waxes seems to be either reducing feather wear, increasing water repellency, providing UV protection and/or possibly repelling lice, mites and other macro-ectoparasites (Elder 1954; Kolattukudy et al. 1987; Montalti and Salibian 2000; Sinninghe Damste et al. 2000; Sweeney et al. 2004). Few of these hypotheses have been adequately tested, and recently Surmacki (2008) ruled out the idea that preen waxes protect carotenoid plumage from bleaching as he failed to find any relationship between preen waxes and reduction in photooxidation by UV light. Although there is some support for a symbiotic relationship between some ectoparasites that feed on the feathers and the production of UG waxes, this hypothesis requires further research (Shawkey et al. 2003; Galvan & Sanz 2006).

Whatever the possible benefits of UG waxes, the composition of secretions varies between species, sexes and temporally (Lambrechts and Hossaert-McKey 2006). For example, the UG secretions of both male and Female mallards (*Anas platyrhynchos*) change in the breeding season with a shift to longer chain esters (Kolattukudy et al. 1987). Similarly, a survey of 20 sandpiper (Family Scolopacidae) species found that birds switched from producing preen waxes consisting of low molecular weight monoesters in the non-breeding season to producing high molecular weight diesters in the breeding season (Reneerkens et al. 2002). Further work found that not only was this change reversed immediately after the clutch fledged, but the switch was predominantly associated only with the sex which incubates (Reneerkens et al. 2002), suggesting that the switch is tightly associated with the incubation and brooding phase of the breeding cycle. Seasonal changes in the profile of UG secretions have also been observed in dark-eyed juncos (*Junco hiemalis*; Soini et al. 2007) and house finches (*Carpodacus mexicanus*; Haribal et al. 2005) suggesting a

general pattern across all birds. The function of seasonal variation in UG products has produced a number of theories. Piersma et al. (1999) initially proposed that the temporary switch from monoesters to diesters in the breeding season facilitated the sexually selected requirement for attractive plumage (i.e., the secretions made the plumage more attractive to prospective mates). This was later refuted by the discovery that diester waxes did not significantly change plumage reflectance and therefore could not increase the feathers 'attractiveness' (Reneerkens and Korsten 2004). The timing of the shift also argues against this 'avian makeup' hypothesis as the diester waxes are produced after the mate choice phase of the breeding cycle (Reneerkens, et al. 2002). Recently, Reneerkens et al. (2007) found that diester preen waxes were predominantly produced only by the incubating sex in species exhibiting uniparental incubation. This finding suggested that the switch to diester waxes is directly related to incubation, and thus that the change in wax composition is due to selection acting on individuals responsible for incubation. Diesters are longer, have more branches and greater numbers of alcohol moieties, properties that mean they are heavier and therefore less volatile than monesters. As the volatility of wax esters is inversely proportional to their size, Reneerkens et al. (2005) suggested that the switch to diester waxes by incubating birds was a mechanism to increase olfactory crypsis against mammalian predators. They discovered that a potential mammalian predator (in this case, a dog) had greater difficulty locating sources of diesters than monoesters. In other words, they proposed that the seasonal switch from monester to diesters by incubating birds was an evolved adaptation to prevent potential predators locating them (and their nests) using chemosensory cues.

To date all studies of UG products have been conducted on continental species of birds (primarily European and North America species). However, until humans



colonised New Zealand, native birds evolved in the complete absence of mammalian predators (Cooper and Millener 1993). The only other predators present in New Zealand were other species of birds (e.g. falcons, owls), which are less likely to use olfaction to locate prey compared to visual or auditory cues. If the switch in UG wax composition is an adaptation to avoid mammalian predation, then New Zealand birds which have been isolated from mammalian predators (Blumstein and Daniel 2005), might be expected to have either lost such anti-predator behaviour or not evolved them in the first place. In other words, if the switch to diester waxes is an anti-predator adaptation to increase olfactory crypsis, New Zealand birds should not exhibit such a change.

In this chapter, I test the predation-based ‘olfactory crypsis’ hypothesis by determining whether a variety of native New Zealand birds switch UG wax composition between the breeding and non-breeding season as found in continental species of birds that evolved with mammalian predators. For comparison, I also analysed the UG wax composition of a variety of introduced species of birds in New Zealand. Over the last 150 years a number of European birds were introduced to New Zealand by acclimation societies (McDowall 1994). These introductions give me the unique opportunity to conduct a comparative analysis between both the native species (no previous evolutionary history with mammalian predators) and introduced species (co-evolved with mammalian predators in their native range) simultaneously in the same environment. By sampling each group of species in both the breeding and non-breeding season I was able to characterise the shifting regimes of UG wax composition, or lack of, in these two groups of birds.

### 3.3 Materials and Methods.

#### *Collection of preen waxes.*

Samples of preen waxes were collected from live birds in both the breeding and non-breeding season. Birds were sampled in Kowhai Bush, Kaikoura and Motuara Island in the Marlborough Sounds, both on the South Island, New Zealand. A variety of endemic and introduced species were sampled in both locations depending on their abundance. The birds were captured using a combination of Potter traps baited with seeds and mist nets. The native species sampled were bellbird (*Anthornis melanura*), fantail (*Rhipidura fuliginosa*), South Island robin (*Petroica a. australis*), and South Island saddleback (*Philesturnus c. carunculatus*). The introduced species sampled were blackbird (*Turdus merula*), chaffinch (*Fringilla coelebs*), dunnock (*Prunella modularis*), goldfinch (*Carduelis carduelis*), greenfinch (*C. chloris*), redpoll (*C. flammea*), house sparrow (*Passer domesticus*) and yellowhammer (*Emberiza citrinella*). The silvereye (*Zosterops lateralis*) is classified as a native New Zealand bird, but as it only colonised from Tasmania in the 19<sup>th</sup> century, it is classified here as a continental species (i.e., one that evolved on a continent, like the introduced species, and has only been in New Zealand for a short time).

The breeding season samples were collected from September-December 2007. This coincides with the breeding season for both the native and introduced species. Male birds were deemed as breeding by identification of an enlarged cloacal protuberance when examined in the hand (e.g. Wolfson, 1952). Females and incubating males were identified as in breeding condition by the presence of a brood

patch. In a few instances individuals were tracked back to a nest, confirming my identification as an individual in breeding condition. The non-breeding season samples were taken in April-May in 2007 and 2008. This is 2-4 months after the breeding season and about 3-4 months before the next breeding season. No brood patches and cloacal protuberances were found on any birds captured in this period. Birds caught in the breeding season with undeveloped brood patches or small protuberances were sampled but because I was unsure of their exact breeding status, and it is unknown at what stage any switch in preen waxes may occur, I excluded these samples from further analyses.

Preen wax samples were collected by gently massaging the papilla of the uropygial gland (figure 3.1). This lead to the excretion of a small droplet of wax and this was then gathered with a clean cotton wool swab. These swabs were immediately placed in coded plastic airtight containers and stored in a refrigerator at 4 °C for no longer than 6 months until extraction and analysis. Each bird was marked for individual identification before release to ensure it was not subsequently resampled.

### ***Sample extraction and Gas Chromatography analysis.***

The waxes were extracted from the cotton swabs with 2 ml of ethyl acetate (Dekker et al. 2000; Reneerkens et al. 2005) and transferred to black-topped tubes. After the addition of 2 ml of phosphate buffered saline the samples were vortexed for 1 min and then centrifuged at 1500 rpm for 5 min at 7 °C to facilitate phase separation. The top layer of ethyl acetate that contains the waxes was then transferred to a new black-topped tube and dried down in nitrogen gas. The resultant residue was made up to solution with 100 µl of ethyl acetate. Samples varying from 1-3 µl were injected into

the gas chromatographer (GC) for analysis. The temperature profile was based on Dekker et al. (2000) and Reneerkens et al. (2005). Samples were injected into the GC and vaporised at 250 °C. The vapour then condensed on the beginning of the column, a process analogous to on-column injection. The temperature profile began with an initial temperature of 70 °C, rising at 20 °C/min to 130 °C. The second part began at 130 °C, rising at 4 °C/min to a final temp of 320°C, which was held for 15 minutes. The detector was a flame ionisation detector (FID) set at 320 °C. The carrier gas used was Nitrogen set at 42 PSI with a flow rate of 20 cc/min.

Palmitoyl ester and stearyl arachidate were used as standards to ensure the column was producing consistent chromatograms throughout the duration of the project. At regular intervals the column was flushed with hexane to reduce cross contamination between samples. The column was a Virian WCOT Fused Silica, 25MX0.32MM ID, coating CP-SIL %CB DF=0.12 provided by AI Scientific. The phosphate buffered saline (PBS), used for was made up with 150 mM sodium chloride and 10 mM sodium dihydrogen orthophosphate with a pH of 7.4.

### ***Interpretation of chromatograms.***

Chromatograms from each sample provided details on the retention times, the area of the each component, and their percentage area of the curve as a whole (figure 3.2, 3.3). The percentage areas were used in subsequent analyses as this controlled for the initial varying concentrations of the samples. It was not feasible to hold initial concentration of wax constant, as the amount of wax collected was variable between individuals and species. The use of percentage area allowed comparisons of

proportional changes in the composition of the waxes within species, and then comparison of these proportional changes between species.

To quantify each lipid profile, I calculated mean retention time, retention time of greatest peak, and number of elution clusters for each species from the chromatograms. I did this for birds in both the breeding and non-breeding season. Mean retention time was estimated by dividing the sum of all retention time and area between 10 and 60 minutes by the number of peaks between 10 and 60 minutes (Figure 3.2, 3.3). This time interval was chosen as it could be repeated on all chromatograms and in all cases this interval encompasses > 98% of the area of the chromatogram. The retention time with the highest peak was ascertained by recording the time of the peak with the greatest area. The clusters were determined qualitatively as being discreet aggregations of peaks, which were repeatedly expressed within a species (Figure 3.2, 3.3).

### ***Statistical analysis.***

An analysis of variance was performed on the mean difference of the mean retention times of each sex, in the breeding and non-breeding season for each individual species (breeding – non-breeding). The predictors used were origin (introduced or native), sex and species. An analysis of variance was also performed on the difference between the times of the greatest peaks of each sex in the breeding and non-breeding season. The same predictors were used as in the first ANOVA. A test of homogeneity of variances was performed to ensure assumptions of the analysis were met.

### 3.4 Results.

Origin was a significant predictor of the mean difference of preen wax composition (ANOVA:  $F = 44.6$ ,  $df = (1,12)$ ,  $p < 0.001$ ), with New Zealand birds having significantly different mean retention times to continental birds (Table 3.1). All continental species had mean retention times that increased in the breeding season compared to that in the non-breeding season, while no seasonal changes were noted in any New Zealand species except for the South Island robin and the South Island fantail (Table 1). The robin was unusual in that the mean retention time was less ( $>6$  mins) in the breeding season than in the non-breeding season, indicating that the preen waxes in this species actually became more volatile in the breeding season. There was a significant difference between species in preen wax composition ( $F = 23.4$ ,  $df = (11, 12)$   $p < 0.001$ ); however, sex was found to be non-significant ( $F = 2.93$ ,  $df = (1, 12)$   $p = 0.11$ ). The ANOVA was rerun, this time using the difference between retention time of greatest peaks as a response. Origin was again a significant predictor of this difference ( $F = 24.7$ ,  $df = (1, 12)$   $p < 0.001$ ). Species was also a significant predictor ( $F = 19.6$ ,  $df = (11, 12)$   $p < 0.001$ ), but again sex was not significant ( $F = 1.44$ ,  $df = (1, 12)$   $p = 0.27$ ). Although using the difference between the retention times of greatest peaks showed a similar pattern to differences between mean retention times, mean retention time was considered a more comprehensive representation of the chromatogram and therefore the volatility of the samples. This is because mean retention time encompassed  $> 98\%$  of the data of each chromatogram, whereas the greatest peak only represented that percentage of the chromatogram that was contained in that peak.

The number of peaks varied from species to species and appeared to differ with origin. With the exception of the blackbird and silvereye, all continental species displayed 3 distinct peaks in their wax profile (figure 3.2). The middle cluster was the largest in most species, but the third cluster, which represented compounds with the longest retention times, became proportionally larger in the breeding season in continental species but were entirely absent in native species (figure 3.3). In contrast, the blackbird had only two distinct clusters, but mean retention times averaged around 50 minutes, a considerably longer time than all of the other continental species (mean retention times 32.9-41.1 mins for all other continental species). Both the robin and saddleback had chromatograms in which no clear clusters could be identified.

With the exception of the house sparrow, in all of the continental species with uniparental female incubation, the female showed a greater positive shift in mean retention time in the breeding season than did the male. Interestingly, in the silvereye and fantail, two species that exhibit bi-parental care, it was the male that showed a greater positive shift in mean retention time during the breeding season.

### **3.5 Discussion.**

I found that continental species change the composition of their preen waxes during the breeding season, whereas the native birds generally did not. All of the continental species exhibited a positive shift such that their mean retention times were greater in the breeding season than in the non-breeding season. As mean retention time was used to characterize overall volatility, this result confirms that during the breeding season, continental birds switch to preen waxes that are less volatile (i.e.,

less “smelly”). In contrast, native species generally showed no, or in some cases a negative shift in the mean retention time, indicating that they are not switching to less volatile waxes in the breeding season, and in the case of the South Island robin, are actually switching to waxes that are more volatile. Sex was found to be non-significant, but this is somewhat confounded by the fact that the difference between sexes varied depending not only the parental care regime of the species, but also on the origin of the species.

Reneerkens et al. (2005) proposed the olfactory crypsis theory on the basis that a reduction in volatility, and therefore locatability is the reason for switching to diesters in the breeding season. The consistent shifting to less volatile secretions in the breeding season by the introduced species, that have evolved with mammalian predation, suggests the phenomenon is connected to predation pressure. Similarly, the fact that the native birds, which evolved without mammalian predation, did not produce less volatile waxes in the breeding season, and in some cases produced more volatile secretions, suggests that the composition of their secretions is under an entirely different set of selective pressures.

Reneerkens et al. (2007) also suggest that parental care regime could be used to predict the secretions of the species using them. All of the continental birds that exhibit uniparental care, with the exception of dunnocks displayed a greater positive shift to less volatile secretions in the incubating sex. The species that used bi-parental care, with the exception of the goldfinch, showed a greater positive shift towards less volatile secretions in the male. These differences could not be tested statistically, but the trend certainly follows that of previous research, and begs for further analysis of these disparities between sexes of different parental care systems.



The native South Island fantail proved to be the exception to the rule by showing a shift to producing less volatile secretions in the breeding season. In the native species used for this study, the South Island fantail is probably the most recent coloniser of New Zealand as it is often considered congeneric with the Australian grey fantail and likely reached New Zealand only in the last few thousand years. Although little is known about the plasticity of the expression of the preen waxes, it is possible that South Island fantails have simply retained the wax shifting characteristics of their ancestors which evolved with mammalian predation in Australia. South Island fantails utilise a bi-parental care system, and similarly to the silveryeye that use a bi-parental care system, the male showed a slightly greater shift to less volatile secretions in the breeding season.

It has been suggested that the production of long chain waxes is to retard the release of active constituents in the waxes (Burger et al. 2004). If this is the case then perhaps the shift need not be as dramatic as illustrated by Reneerkens et al. (2002) with a complete shift to a secretion almost exclusively composed of diesters. Perhaps the slight increase in just a few key long chain waxes could act to impede the release of the remaining volatile components, resulting in a similar outcome of reducing olfactory cues. The mechanisms of olfactory crypsis seem to require that the boiling point of the secretion need only be slightly adjusted to dramatically affect the evaporation/sublimation of a secretion in an environment where the ambient temperature was predictable and stable. Particularly if the cost of producing less volatile secretions is high, an individual might want to be as conservative as possible when determining the lipid profile of its secretions. It may be the case that passerines are reducing the volatility of their secretions just enough to reduce locatability of their

nest a sufficient amount to outweigh the costs of producing the greater molecular weight components.

The case of the South Island robin was particularly interesting in that they actually produced secretions that were considerably more volatile in the breeding season. Without selective pressure from mammalian predation, New Zealand birds can be thought to be producing preen waxes which are effectively the default for what is required for ecophysiological functionality. This would lead one to predict that the most economical regime would be to produce preen waxes of a consistent composition throughout the year. This was generally the case in the rest of the native species. The fact that South Island robins displayed a shift in their waxes, suggests that there is selective pressure, particularly around the breeding season, to produce waxes of a different, and more volatile composition. None of the other native species exhibit such a dramatic increase in volatility in the breeding season, which reduces the probability that the selection pressure is an environmental one (increase in UV exposure, change in food availability). There is a possibility for preen waxes to take a role in intraspecific communication, for instance the use of biochemical cues to aid mate choice. Olfactory communication in birds is not unheard of, with crested auklets (*Aethia cristatella*) being documented as utilising citrus-like odorants to attract and retain mates (Hagelin et al. 2003). There is also the possibility that a measure of compatibility can be determined from the waxes, similar to the MHC complexes that are famously described in the “white t-shirt” experiment by Wedekind et al. (1995). This could explain why both sexes exhibit the switch to a similar degree, as an internal template may be required to facilitate such a judgment of compatibility.

My findings not only support the hypothesis of olfactory crypsis, but I have also presented the possibility that the role of preen waxes is far more complex than

previously thought. The next step would seem to be to analyse the waxes of these species using a Gas chromatography-Mass spectrophotometry technique, which would allow a more comprehensive assessment of how the waxes change. Secondly, it would be desirable to perform a similar comparative study on other predator free island groups, to see if this pattern holds for all species that have evolved without mammalian predation. This would possibly illuminate other anomalies, like the South Island robin, that seem to have evolved preen waxes that have functions other than that of feather protection and maintenance.

Finally, my results suggest a possible reason why native New Zealand birds have fared so poorly since the introduction of exotic predatory mammals. Since the colonisation of New Zealand, firstly by Maori in the 1300s, and then by Europeans beginning in the 18<sup>th</sup> century, more than 40% of the terrestrial bird fauna has become extinct, and many of the remaining surviving endemics are threatened with extinction (Holdaway 1989). This contrasts with the stunning success of introduced species of European birds, and recent self-colonising species from Australia (e.g. silvereye, welcome swallow), which are often more abundant than in their native range. It is possible that this difference may stem in part of the differing vulnerabilities of the two groups of species to mammalian predators. Although there are a number of other behavioural and life history traits that differ between native New Zealand birds and continental species, some that might explain some of the difference in their ability to cope with introduced mammalian predators, my findings suggest it would be worth considering the absence of a switch to less volatile preen waxes during the breeding season. If this makes birds more vulnerable to detection, then native species may be particularly prone to predation by mammals. Native New Zealand birds may be so at risk of extinction simply because they are smellier!

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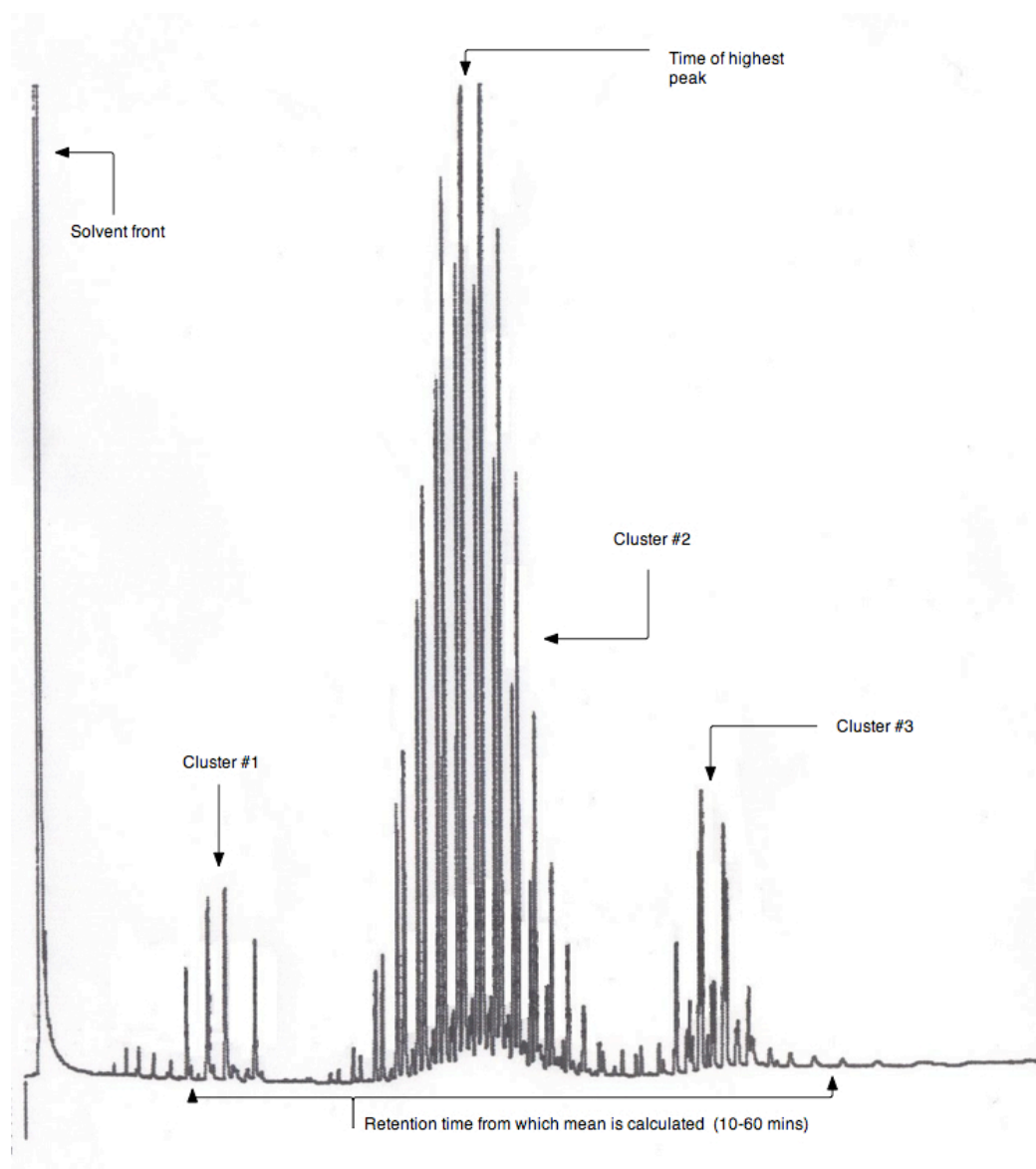
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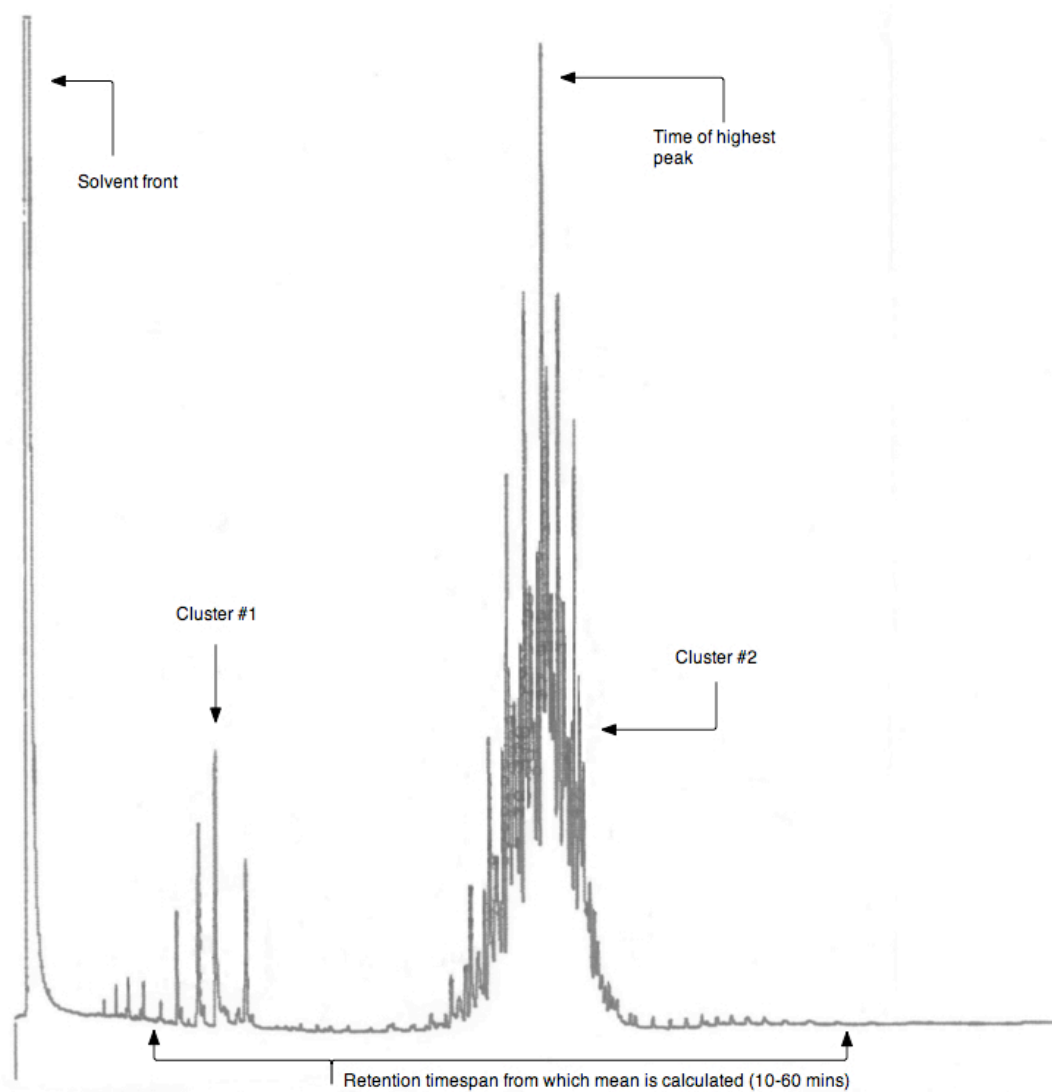




**Figure 3.1.** Sampling of preen waxes from the uropygial gland of a dunnock (*Prunella modularis*) in the field, Kaikoura.



**Figure 3.2.** Typical chromatogram of a breeding (continental) greenfinch *Carduelis chloris*.



**Figure 3.3** Typical chromatogram for a breeding (island) bellbird *Anthornis melanura*.

**Table 3.1.** Table showing the mean retention times of introduced and native species in the breeding and non-breeding season and the difference between these means as calculated from chromatograms produced by gas chromatography.

Species	Sex	Breeding season (mins)	Non-breeding season (mins)	Difference (Breeding-non breeding)
<b><u>European</u></b>				
Blackbird	<i>M</i>	46.9	44.84	2.06
	<i>F</i>	50.47	47.91	2.56
Chaffinch	<i>M</i>	36.25	34.87	1.38
	<i>F</i>	37.15	34.27	2.88
Dunnock	<i>M</i>	37.95	35.75	2.2
	<i>F</i>	38.12	35.75	2.37
Goldfinch	<i>M</i>	35.33	34.65	0.68
	<i>F</i>	37.51	35.33	2.18
Greenfinch	<i>M</i>	36.1	35.36	0.74
	<i>F</i>	36.53	36.03	1.5
House sparrow	<i>M</i>	37.4	36.44	0.96
	<i>F</i>	36.73	36.96	-0.23
Redpoll	<i>M</i>	35.94	35.4	0.54
	<i>F</i>	36.14	35.24	0.9
Silvereye	<i>M</i>	41.42	40.09	1.33
	<i>F</i>	40.37	40.09	0.28
Yellow hammer	<i>M</i>	38.18	38.17	0.01
	<i>F</i>	40.62	38.9	1.72
<b><u>New Zealand</u></b>				
Bellbird	<i>M</i>	39.17	39	0.17
	<i>F</i>	41.25	39.97	1.28
Fantail	<i>M</i>	40.14	36.34	3.8
	<i>F</i>	39.94	36.34	3.6
Robin	<i>M</i>	27.94	35.43	-7.49
	<i>F</i>	29.9	35.94	-6.04
Saddleback	<i>M</i>	26.92	26.94	-0.02
	<i>F</i>	27.88	28.37	-0.5

## **Chapter 4**

# **An investigation of evolutionary changes in colouration, size and morphology of insular passerines.**

### **4.1 Abstract.**

The changes in size and colour in insular birds compared to their continental relatives has intrigued scientists for centuries but there have been few systematic studies using robust phylogenetic methods. My study investigated body and bill size changes in isolated island passerines compared to their continental counterparts. I also looked at changes in the extent of carotenoid and melanin pigmentation, and whether this resulted in brighter or duller island forms. Using the pairwise comparative method, I found that island birds are significantly larger than their continental counterparts and incorporated significantly greater amount of melanins into their plumage. I also found island species to be significantly duller compared to their continental counterparts due to the combined effects of reduced carotenoids and increased melanins. Finally, I found that bill morphology changed with island species having significantly larger bills. These results confirm that island environments affect the evolution of both the appearance and morphology of a species. However, further research into the intricacies of these patterns is needed to determine exactly why island environments have induced such dramatic changes.

## 4.2 Introduction.

Even to a casual observer, birds on islands seem different from birds in large continental regions. Not only do birds on islands seem tame and oblivious to the dangers that humans pose (and their commensals such as rats), but they often differ in colour and size from continental species. Indeed, it was these differences that so intrigued Charles Darwin in the Galapagos Islands and played a key role in his later development of ideas on speciation and natural selection. Why is it that plants and animals often differ from their counterparts on nearby continental areas? Trying to understand the reasons for why organisms differ between islands and continents first requires a detailed knowledge of the changes observed on islands.

One of the most common patterns observed in island animals is a change in body size. Amongst the earliest observations is of small-bodied continental species having larger insular counterparts (van Valen 1965; Lomolino 1985). It has also been observed that small insular passerine species are in fact larger than their continental counterparts (Grant 1965; Clegg and Owens 2002). A more elegant observation, coined the ‘island rule’ by van Valen (1973), concerned the pattern of small mammals on islands becoming larger and large mammals becoming smaller on islands. A tendency towards dwarfism of large species and gigantism of smaller species on isolated islands has been explored in insects, reptiles and birds (Clegg and Owens 2002; Meiri 2007; Palmer 2002). One causative argument for these observations is that smaller species increase in size to sequester more resources and increase their metabolic efficiency (Meiri et al. 2008). Large species, on the other hand, exhibit a reduction in size to decrease resource requirements and maximise reproductive output. The resulting ‘medium’ body size can be thought of as the optimum size for

an organism evolving with little predation, and only the pressures of resource availability and competition affecting their evolution. However, patterns opposite to this have been found in lizards in which small species on islands became smaller than their continental counterparts, while the larger species increased in size (Meiri 2007).

The 'island rule' was previously thought not to apply to birds, but Clegg and Owens (2002) attributed this to the disproportionately large amount of passerines used in those analysis. When birds from a wide range of orders were compiled into a more comprehensive analysis, as in that of Clegg and Owens (2002), the 'island rule' was realised. Similar to changes in body size, there has been some argument as to the patterns in bill size and morphology changes in island species. These range from the assessment that bill size has ubiquitously increased in island forms (Mayr 1942; Grant 1965; Case 1978; Scott et al. 2003), to the suggestion that they follow the 'island rule' (Clegg and Owens 2002), where large billed species evolve smaller bills on islands, and small billed species evolve larger bills upon island isolation. Apart from morphological changes in body size and bill size, there is some evidence to suggest that the colours of birds also differ between island species and their continental counterparts. Two groups of pigments are responsible for some of the colour of feathers: carotenoids which give the yellow, orange and red colours, and melanins, which give the brown and black colours (white is due to the absence of any pigment). Mayr (1942) was the first to suggest that the reduction in species diversity on islands lead to a relaxation of isolating mechanisms (i.e., colours that identify a species to conspecifics and thus prevent hybridisation), and could account for the loss of M colour in island species. More recently, Doucet et al. (2004) proposed that island plumage variation might be more strongly influenced by natural selection, than sexual selection. This could lead to increases in the more functional pigments, like melanins,

with a reduction in more costly pigments, like carotenoids. The problem arises when attempting to distinguish between a simple relaxation in sexually selected traits, with increases of melanins as a by-product, and progressive selection for more melanin pigmentation through natural selection.

Carotenoids were first thought to be limited in their expression by environmental availability alone (Endler 1980). However, parasitism is also now known to limit the expression of carotenoid pigmentation (Milinski and Bakker 1990). This could result in individuals in depauperate habitats (perhaps such as isolated islands) having an impaired immune function and thereby exhibiting less carotenoids. Matson (2006) found no reduction in immune function of insular birds, and in fact found that several indices increased. If carotenoid expression were controlled by immune functions and resource availability alone, then assuming the precursors for their production are equally distributed amongst islands and continents, one would predict no difference in carotenoid pigmentation between island and continental species. The expression of carotenoid pigmentation in island species is most likely controlled by combinations of various environmental and genetic factors; the variation of which between island and continental environments, possibly results in the different patterns of colouration.

Melanins are pigments associated with the dark colouration of plumage and have long known to increase resistance of abrasive wear (Barrowclough and Sibley 1980; Lee and Grant 1986). They have also been correlated with reductions in preening effort (Roulin 2007), meaning there is a fitness advantage to their incorporation into an individual's plumage. For an individual to increase their use of melanins could mean that the novel environment is imposing a prerequisite for more robust plumage. Or it could be that they need to reduce the time spent on feather



maintenance due to the different time budgets required by a different environment. Perhaps differences between islands and continents in the advantages and disadvantages of melanin level could explain the change in expression of this pigment.

Despite the long interest in the question of how birds change on islands, and why this might occur, there have been surprisingly few systematic attempts to survey birds on a wide range of islands and that control for potentially confounding affects such as phylogeny, island type, and type of colour change. In this chapter, I use the pair-wise comparative to study potential changes in body size, bill size and plumage colouration across a wide variety of passerine birds on about 15 islands or island groups. My objective is to quantify and test whether there are indeed consistent changes in body size and bill size in island birds. I also test whether island birds exhibit a change in colour through either a reduction in carotenoid and/or melanin pigmentation. Although my study seeks to investigate the ways the morphology and size of insular species have changed, I also speculate upon the reasons for these changes.

### **4.3 Methods.**

A pairwise comparative method was used similar to that described by Møller and Birkhead (1992). This involved selecting a series of “species pairs” where one member of the pair was a continental species while the other member of the pair was its closest phylogenetic island relative. Each pair was considered a single and independent evolutionary event (i.e., any change in colour or size in one species pair was assumed to be independent of that in a second species pair) and any difference in

morphology or colour between the two members of a pair were thus assumed to have evolved since they shared a common ancestor.

I focused my assessment of changes in morphology and colour to those species that occur only on small oceanic islands. This included birds on the following islands or island groups: Azores, Canary, Cape Verde, Sao Tome and Principe, Mauritius, Seychelles, Reunion, Lord Howe, Norfolk, Chatham, South Georgia, Juan Fernandez, Galapagos, Revillagigedo, and Guadalupe. Thus I did not consider species that occur on large continental islands such as Japan, New Guinea, Madagascar, or New Zealand, all of which were once connected to continental landmasses and contain species that arrived on the island *in situ* (i.e., present when island formed) or through colonisation. Instead, I only used islands that are oceanic and were never directly connected to any continental region. As all birds that occur on these islands must be the product of colonisation (unlike that of the larger continental islands), any changes in morphology are likely to have arisen since the time that an island was reached by the ancestral population. All the islands in my sample are also relatively small (34.6 - 7880 km<sup>2</sup>, average - 2558 km<sup>2</sup>) which avoided potentially confounding problems of island size, such as would occur if, for example, one was comparing changes in morphology of birds between Africa and Madagascar versus Africa and the Seychelles. The large size of Madagascar compared to the Seychelles means that any differences found would be hard to differentiate due to an “island-continental effect” or to a “big-small island effect.”

Species pairs were selected in the following manner. First, I made a list of all passerine species that occurred in each island group. I restricted my sample to passerine birds to avoid the inclusion of other island birds, such as seabirds, which disperse widely after breeding and thus are not necessarily isolated from populations

of conspecifics elsewhere. For each island species, I then paired it up with its closest relative on the nearest continental area. For example, birds on the Revillagigedo Islands were paired up with their closest phylogenetic relatives in Mexico, while birds on the Azores were paired up with birds in south-western Europe and north-east Africa. Each species pair involved either only a congener (i.e., both island and continental birds were classified in the same genus) or a conspecific (i.e., both island and continental birds were classified as the same species but different subspecies). I did not use birds that were the same subspecies on both the island and continental populations as these are likely to have been the product of very recent colonisations. I also did not use island species in endemic genera as these could not always be matched up unequivocally with a continental species. Thus the changes in morphology and colour I tested are those that are likely to appear with the formation of new subspecies or species on islands, but not over the greater periods of time that are involved in the formation of endemic genera or higher taxa. Information on the taxonomy of island birds and their closest continental relatives were obtained from literature (Dickinson 2003). In some cases an island group might have more than one subspecies present (e.g. blue tits in the Canary Islands); in this situation I selected only one subspecies to pair and this was the one in which had the most information for a given attribute (e.g. body mass). As each statistical test was independent of the others, different pairs were sometimes chosen for each attribute depending on the information available. For instance, if one species had detailed information on dimensions, but none on colour, and another pair had a description of appearance, but no data on size or weight, a different pair was used to test each attribute. In all cases, the continental species always remains the same, and only one species pair was used for a given test to avoid pseudoreplication.

### *Assessment of pairs.*

I relied on published literature for all my estimates of body size (mass, total length, wing length, bill size) and I used colour descriptions and colour plates in a variety of field guides to estimate differences in carotenoid and melanin colouration in each species pairs. For each species pair comparison, I compared the island form with the continental form and gave it a positive or negative score when there was either an increase or decrease in expression of a trait relative to the island form, respectively. For example, a positive score in body size for a particular species pair meant that the island form had increased with respect to its continental counterpart. Overall, I used a total of 98 species pairs in my analysis (see appendix 4.1).

### *Size.*

Body size was reported in the literature as either body mass or body length. For the assessment of size changes I analysed each measure separately but then also combined both in a general “body size” estimate. Thus I combined species pairs in which only body length data was available, with species pairs in which only body mass data was available. This did not allow for any estimation of how much changed occurred, just whether a change occurred or not, which can still be tested using a sign test. If both mass and length data were available, I used mass as the principal measure. This was to reduce the confounding effect of insular avian species reducing the size of the feathers associated with flight (wings & tail) (Diamond 1981). However, if only length was given then this was used. In some cases the author provided their own assessment of size stating, “subspecies A is larger than subspecies

B”, or that “the island form is larger”. Qualitative evaluations like these were used when no numerical data was available. A similar system was used for assessment of changes in bill morphology. Length was the most commonly available dimension, but sometimes authors gave information on the shape of the bill. Numerical data were used in preference to author’s notes with greater lengths resulting in an “increase” score. However, when author’s notes were used, such assessments as ‘more swollen’, ‘broader’ or simply ‘larger’ also all rated a score of an ‘increase’.

### ***Colour.***

The first characteristic of colour to be assessed was carotenoid pigmentation. The principal assessment of an increase or decrease in carotenoids was surface area. Any reduction or increase in area of the bird’s plumage exhibiting carotenoids resulted in the scoring of an increase or decrease in carotenoids, respectively. If the area of carotenoid pigmentation remained the same but the colour changed from orange to yellow, or red to pink then this was also considered a reduction in carotenoid pigmentation. This method would be confounded if area were to be reduced, but with a positive change up the spectrum, for example from yellow to red, but in my analysis this situation never occurred. The third possibility was a deepening, or increase in intensity of carotenoid pigmentation. A distinction had to be made as to whether this was a result of increased carotenoids, or additional melanins. If the carotenoids appeared just darker then this was assumed to be increased melanins. If the carotenoids became more intense, or redder and brighter, then this was taken to be more carotenoids. A note of caution is needed here that my assessment of colour is based on my own human sense of vision, and it is well known that birds can see in the

UV range (Hausmann 2002). Thus, the colours we see may not be what the birds themselves perceive. However, my results should still be indicative of any changes in colouration that occur in the “visible” spectrum.

The second colour characteristic to be assessed was melanin pigmentation. Similar to the carotenoid assessment, if there was a greater surface area of the bird containing melanin pigmentation, then I considered this to be an increase in melanin pigmentation, and vice-versa. If no pictures were available, but there were author’s notes about appearance, phrases such as, ‘more heavily streaked’, were taken to be indicative of more melanin pigmentation. Also the description of ‘darker than’ was assumed to indicate an increase in melanins.

The final assessment of colouration was an overall estimation of ‘dullness’. This was a subjective measure that ranked each member of a species pair as either more or less conspicuous. I considered dullness as the opposite of conspicuous as far as my perspective and visual range was concerned. A conspicuous species was characterised by highly contrasting, bright, and/or iridescent plumage. The juxtaposition of contrasting colours, such as black and white, was a common form of contrast. Any loss of iridescence was also seen as an increase in ‘dullness’ as was loss of contrast. The reduction in carotenoid colouration was also considered an increase in ‘dullness’ but the measure of ‘dullness’ was an inclusive assessment that incorporated changes in both carotenoids and melanins with any differences in contrast that coincided with those changes. Therefore a judgment of ‘dullness’ was made upon the assessment of changes in carotenoids and melanins together to decide whether their combined differences resulted in a ‘duller’ bird. When pictures were not available, I used the author’s comments to assess any changes, with authors

commonly making reference to a particular species being ‘duller’ or ‘brighter’ than another.

### ***Statistical analysis.***

A sign test was performed substituting + and – for increase and decrease respectively. Scores of no change were not counted. The statistical test was performed on the data using a critical value of  $p = 0.05$ . As I was not able to obtain all information for each species pair, my sample size varied from one measurement to the next.

## **4.4 Results.**

### ***Changes in body size***

Body size, an inclusive measure including mass and dimensions, of birds on islands increased more often than it decreased. Out of 63 species pairs with information on body size, 43 showed an increase on islands while only 20 island species showed a decrease in size compared to their continental relatives (figure 4.1). This is significantly more frequent than expected (figure 4.1; sign test:  $p = 0.005$ ,  $n = 63$ ). A further body size analysis was performed, this time on the mass data only. In this case 18 of the 25 pairs were seen to increase in mass, which was significantly more than expected (figure 4.1; sign test:  $p = 0.043$ ,  $n = 25$ ). The mass of continental species where available, was log transformed and then plotted against their proportional change in mass in the island species (figure 4.2). A regression was performed on

these changes, which was insignificant ( $F = 0.42$ ,  $df = (1, 23)$ ,  $p = 0.52$ ), indicating no relationship between the size of the continental species, and the degree or direction of changes in mass.

Bill size also increased significantly more often on islands with 40 island species showing an increase in bill size over their continental relatives, and only 7 species showing a decrease in bill size on islands (figure 4.1; sign test:  $p < 0.001$ ,  $n = 47$ ). Wing length showed a tendency to decrease on islands (17 island species had shorter wings while only 8 species had longer wings) but this difference was not significant (figure 4.1; sign test:  $p = 0.1$ ,  $n = 25$ ).

### ***Changes in colouration***

The degree of carotenoid colouration decreased in 38 island species and increased in 25 island species but this trend for less orange and red colouration in island birds was not quite significant (figure 4.1, 4.3; sign test:  $p = 0.1$ ,  $n = 63$ ). In contrast, melanin pigmentation was significantly more common in island species with 50 species showing an increase and only 20 species showing a decrease in melanin pigmentation in the island species (figure 4.1, 4.4;  $p < 0.001$ ,  $n = 70$ ). Finally, the ‘dullness’ of island forms also increased significantly with 55 species becoming “duller” and 24 species becoming “brighter” on islands compared to their continental relatives (figure 4.1, 4.5;  $p < 0.001$ ,  $n = 79$ ).



## 4.5 Discussion.

I found that passerine birds on islands were more likely to increase than decrease in size relative to their continental counterparts. Similarly, bill size was found to be greater in island species than their closest continental relatives, and there was a weak trend for wing length to decrease on islands, although this was not significant. The plumage of island birds also differed from their continental counterparts and was more likely to be duller and show an increase in melanin-based plumage. Overall, my systematic survey using robust phylogenetic methods of analysis confirms that island birds are indeed different from continental birds. Island environments, and the selection pressures birds face on islands, must be favouring the changes I observed.

Many of the mixed results amongst previous studies attempting to find patterns of size change in island birds can be attributed to three things: 1. using wingspan to represent changes in body size, 2. using a restricted selection of species (predominantly passerines), and 3. by disregarding phylogenetic effects. At least some avian species on isolated islands are known to lose their predator escape responses, including a reduced ability to fly, the loss of migratory behaviour, or even flightlessness (Diamond 1981). With the loss of flight comes a concomitant reduction of the flight feathers, and so birds on isolated islands could be predicted to have smaller wings than their continental counterparts, independent of body size. The feathers associated with flight, namely wings and tail, may be under differing selective pressures to the mass, and using length and wingspan to assess size differences can overlook important patterns.

Clegg and Owens (2002) suggest that passerine species, which are generally small-bodied species, present only half of the 'island rule'. So if one is searching for

changes that will correspond to the island rule, but uses a disproportionately large number of passerines in the analysis, they will more than likely find that the trend is only an increase in size, representing only one side of the dichotomy of the rule. My examination of proportional changes in mass showed no relationship between initial mass of continental form and magnitude or direction of any changes in mass. This indicates that the size range of species used in my study represented only those that were selected to increase in size. This presents the possibility that ‘medium’ body size, or the size to which island environments appear to be selecting island birds to achieve, is greater than the largest species used in my study. The difference between a large passerine (around 150 g) and a medium sized ratite (around 7000 g) is an order of magnitude, thus one can imagine such aspects of island ecology as area and food availability, would have vastly different effects across all avian species.

Unlike most previous studies, my study utilised robust phylogenetic methodology to statistically test for differences between island and continental birds. I also only used pairs of species in which the island species had relatively recently colonised an island, at least as indicated by its taxonomic classification at the congeneric or subspecific level, and thereby minimised any effects by other factors that might confound comparisons over vast periods of time, such as with the inclusion of endemic insular genera or families. This does not mean that the patterns I found do not apply to such birds, only that it is possible other factors might mask the pattern, and it is difficult to determine exactly how such species changed without having a continental relative to use as a comparison. I also restricted my analysis to only small isolated, often volcanic, islands in which colonisation is the only basis for a species occurrence. Continental fragments, such as Madagascar and New Zealand were not used as islands as they are ecologically more similar to continental systems, at least in

their large size, than island systems. Again this does not mean the patterns I found on smaller islands do not apply to larger islands (indeed, I used New Zealand as an island example in chapters 2 and 3), only that I wanted to avoid any confounding effects of island size on my results. Previous workers have often disregarded these effects, resulting in a bewildering variety of conclusions on how islands influence the evolution of birds. I think my simpler approach helps to isolate those mechanisms that operate in insular populations, which can then be extended to other island systems.

Mayr (1942) proposed the 'founder effects model' where the changes in the genetic makeup of an island population were a direct result of the reduced genetic representation of the few founder individuals and subsequent gene loss upon island isolation. Island evolution may be affecting birds in slightly different ways to non-volant animals due to the ability of birds to fly, and the consistent pattern of a reduction of this ability with increased body size. One may consider the situation where potential avian island immigrants are confined by a maximum size, where by the smallest individuals can emigrate to isolated islands, but the largest individuals, of which most are flightless, are unable make it to islands of any considerable isolation. This could mean that avian island colonisers represent a specific type of individual, perhaps those of small body size, and a predisposition to long ranged dispersal. Populations of mammals and other non-volant species that are isolated on island chains or continental fragments may be affected in different ways than populations of birds on oceanic, often volcanic islands by the parameters postulated by a founder effects model. This is because in these cases, isolation is generally the result of marooning, not colonisation. The isolated individuals resulting from marooning events are probably more phenotypically and genotypically variable than populations

resulting from colonisations as the passive isolation due to marooning is inherently more random. This means that marooned individuals do not necessarily represent those with traits that may predispose them to be successful colonisers. If this is the case, then the selection pressures upon island species will have fundamentally different effects depending on whether they are mammals or birds due in part to the different variability of the founder populations. Although Grant (2002) explains how the ‘founder effects model’ has taken a theoretical and empirical battering over the years, its implications for explaining differences in insular evolution between mammals and birds are still very important.

Bill size was seen to increase significantly on islands, but this interpretation of size relies heavily on length, whereas all dimensions of a bird’s bill contribute to its function. Niche expansion was proposed by Scott et al. (2003) to explain the increase in body and bill size of insular silvereyes (*Zosterops lateralis*). An increase in the breadth of foraging behaviours in insular birds, but also more foraging specialists than would be expected by chance, led to the suggestion that niche expansion although important to insular evolution, cannot alone explain changes in size and bill morphology. Unless a consistent pattern of ecological similarities could be found among the islands used in my study, it is hard to link the changes I found in bill size to changes in feeding behaviours alone. There is the possibility that the increase in bill size is simply a non-adaptive consequence of the increased body size exhibited by island species. I was not able to examine proportional changes in bill size, by controlling for body size, as the required data were simply not available. Considering, however, that my study showed bill size increasing more often than body size, or mass alone (figure 4.1), perhaps indicates that bill size is possibly under a different selective pressure than body size. One would also assume if bill size were an

allometric outcome of increased body size, then all dimensions, including wingspan, would be increasing in a similar way. As my study has shown, this is not the case. The consistent changes of bill size and morphology of insular passerines, in conjunction with the increase in body size, mean that there must be more similarities between the environments in which these changes are displayed than there are differences.

Carotenoid pigmentation appeared to decrease in the island species, and although this was not significant, it followed my predictions. The proposed relaxation of sexual selection may account for the reduction in carotenoid pigmentation, which is often a sexually selected trait (Doucet et al. 2004). The effect of insular evolution may be different on species for which visual signalling is their primary means of conspecific communication (e.g. signalling quality to potential mates). Such individuals may exhibit carotenoid pigmentation as a secondary sexual characteristic. My study used passerines, which are well-known for their complex songs, and might be better able to forfeit the use of carotenoid pigmentation, as this can be compensated with their vocalisations. In this regard it would be interesting to extend my study to examine whether the structure and complexity of songs have also changed on islands.

Carotenoids also have important roles in vitamin A production and immunocompetence, including free-radicle removal and enhancement of immune function (Hill 1999). These functions, one would assume, are equally as important to island species as they are to continental forms. There could therefore be a reallocation of these compounds when availability becomes limited, from integumentary pigments, to more important physiological roles as described above. As has been alluded to earlier, it is hard to pinpoint exact mechanisms for these

reductions, but the results indicate that insularity is affecting more than just size and morphology.

The significant increase in melanin pigmentation observed in the island species is possibly a response to the ‘harsher’ environment of islands as proposed by Doucet et al. (2004). Melanins are known to increase the abrasion resistance of feathers (Bonser 1995), and their increased expression on islands may be an adaptive response for the less forgiving habitats found on islands. Increased UV exposure, increased salinity of the air, and less sheltered habitats are all possible contributors to the ‘harshness’ of an island, and therefore the need for more robust plumage. Allowance for differences in the environments from which island species originate must be taken into consideration. If the harshness of islands is indeed a driving factor in plumage evolution, one might observe a gradient of melanin use by island species, depending the difference in ‘harshness’ between their continental origins and their new island home. Again this requires further research not only in to mechanisms of these changes, but into the variables that contribute to an island’s ‘harshness’ and how this ‘harshness’ affects other aspects of avian evolution.

The most common pattern I observed was for a general increase in “dullness” in island species, but this explanation simplifies what was a more complex phenomenon. Not all of the continental species were particularly conspicuous, but their island counterparts still exhibited differences that resulted in ultimately ‘duller’ island forms. In a number of species already quite dull brown, there was an almost ubiquitous reduction in the buff to brown colouration between the other darker markings. Warm reddy browns were often replaced with either greys, or darker blacker browns.

Although not tested in any way, there also seemed a strong pattern of the adults of island species retaining the appearance of their juvenile forms. This is most

evident in island birds having reduced expression of their secondary sexual characteristics, like the conspicuous markings that allow one to easily differentiate between adults and juveniles. The increased streaking in a number of island species also meant that the adults became more similar to their more cryptic juvenile forms. However, the lack of juvenile plumages being depicted in many of the field guides I used limited my ability to test if this pattern existed more generally, and it needs to be investigated further.

The significant change in the colours observed in island species should be treated with some caution. The pattern I found was highly significant, but is based on the appearance of birds through human eyes, which are insensitive to the UV spectrum. Hausmann et al. (2002) found that visual signals utilising UV contrast in avian species are important to sexual displays. Therefore without a comprehensive spectro-photometric analysis, it would be hard to assess whether colouration associated with sexual displays is increasing or decreasing on islands. What can be confirmed however, is that birds are not only showing a predictable pattern of increased size, but are also showing interesting patterns of plumage modification that are exhibited across a vast range of species that originated from a highly variable set of continental environments.

A large number of theories have been formulated to explain the patterns of change in size of island birds. Many of these present a situation where a combination of effects from genetic to environmental, coalesce, resulting in birds exhibiting consistent patterns of changes in size. My study presents a situation where changes in size appear to be occurring in tandem with consistent shifts in plumage colouration. This could mean that size, morphology and colouration are under the same selective pressures, or that these patterns present a fascinating coincidence. What needs to be

done to find out exactly what is changing in island birds, is a comprehensive analysis using spectro-photometric techniques on live island specimens, along side a full assessment of changes in bill morphology and mass of live birds. This approach is far superior to using museum skins, or field guides, as it will allow the additional investigation of how the UV spectrum might be important to island species, and allow calculation of proportional changes in the morphology of island birds.



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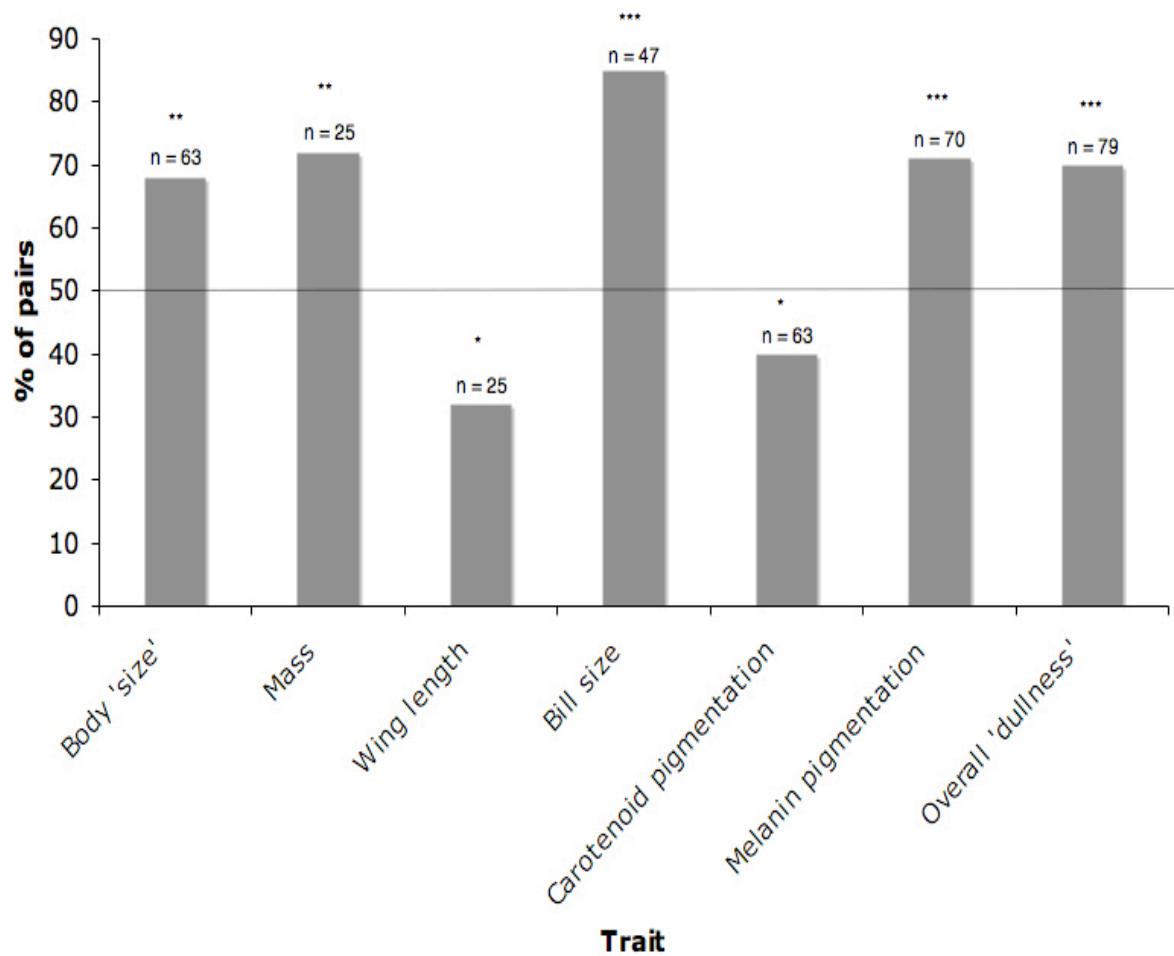
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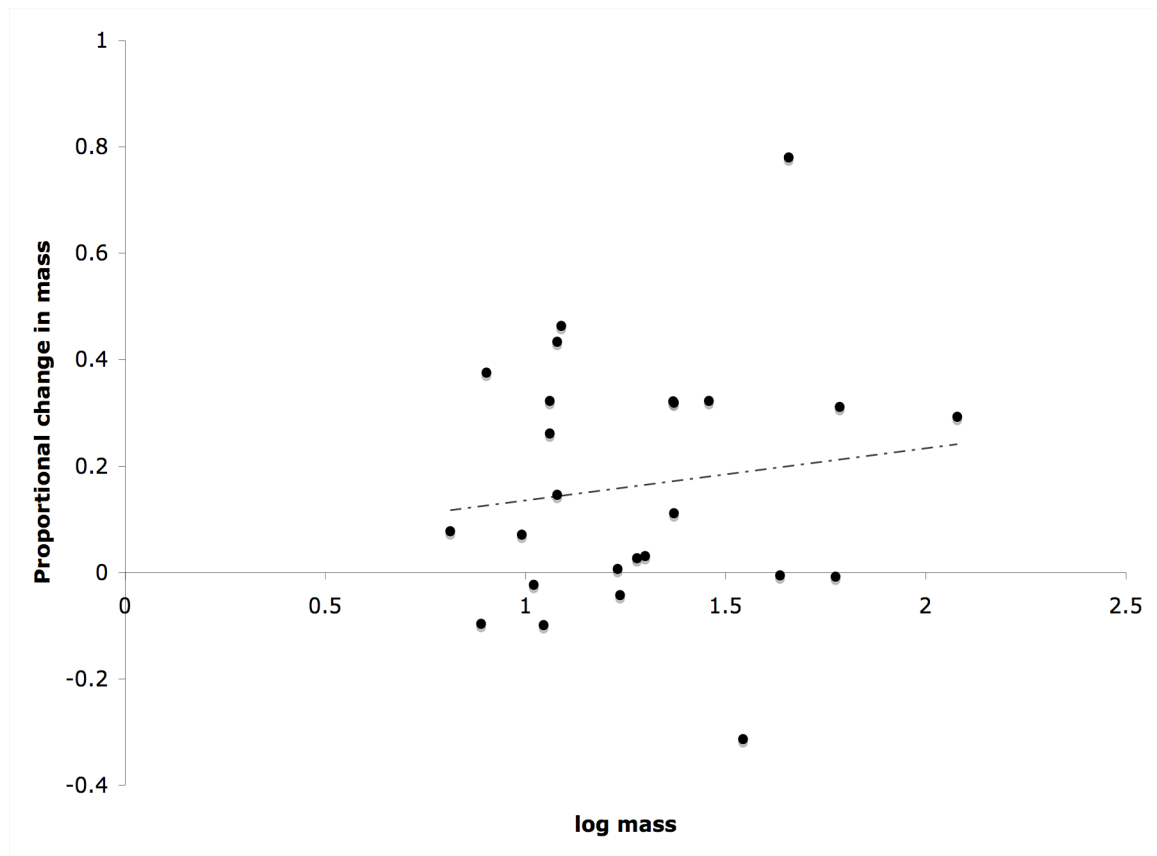
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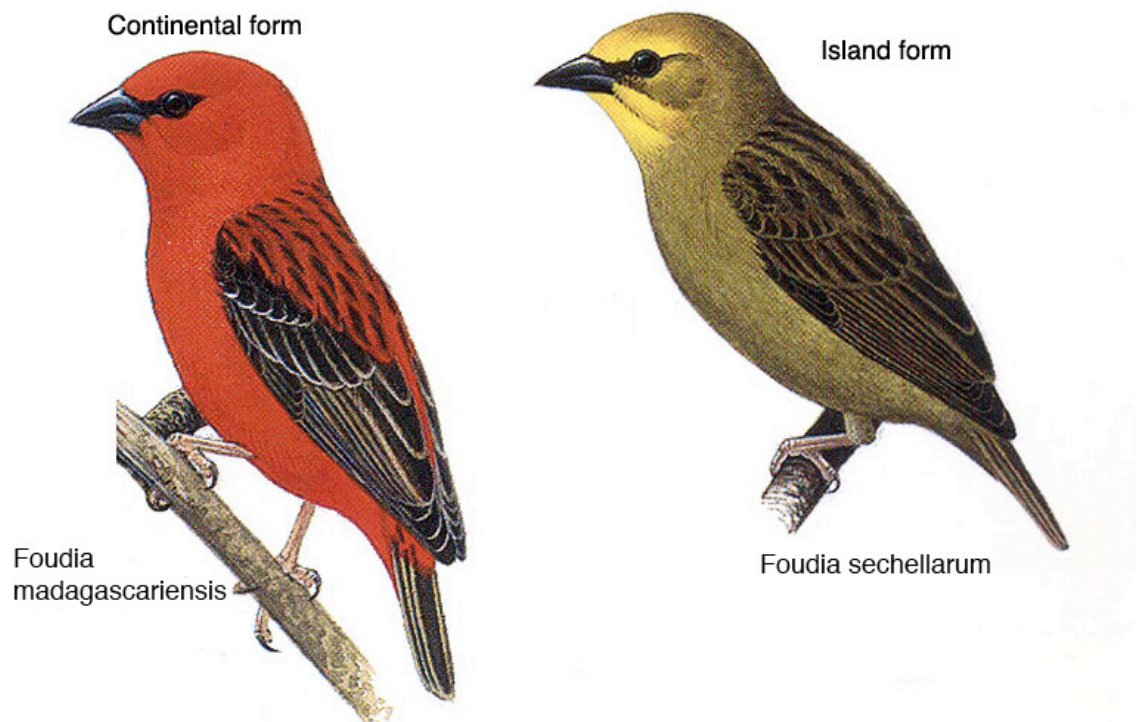


**Figure 4.1.** Graph showing the percentage of species pairs in which the island species showed an increase in the specified trait. (Body 'size' was estimated using mass, length, and author's notes to come to approximation of which species was larger. Mass used weight data only). The line at 50% represents the percentage of pairs expected to show an increase by chance; the null hypothesis. (\* =  $p \leq 0.1$ , \*\* =  $p \leq 0.05$ , \*\*\* =  $p < 0.000$ ).

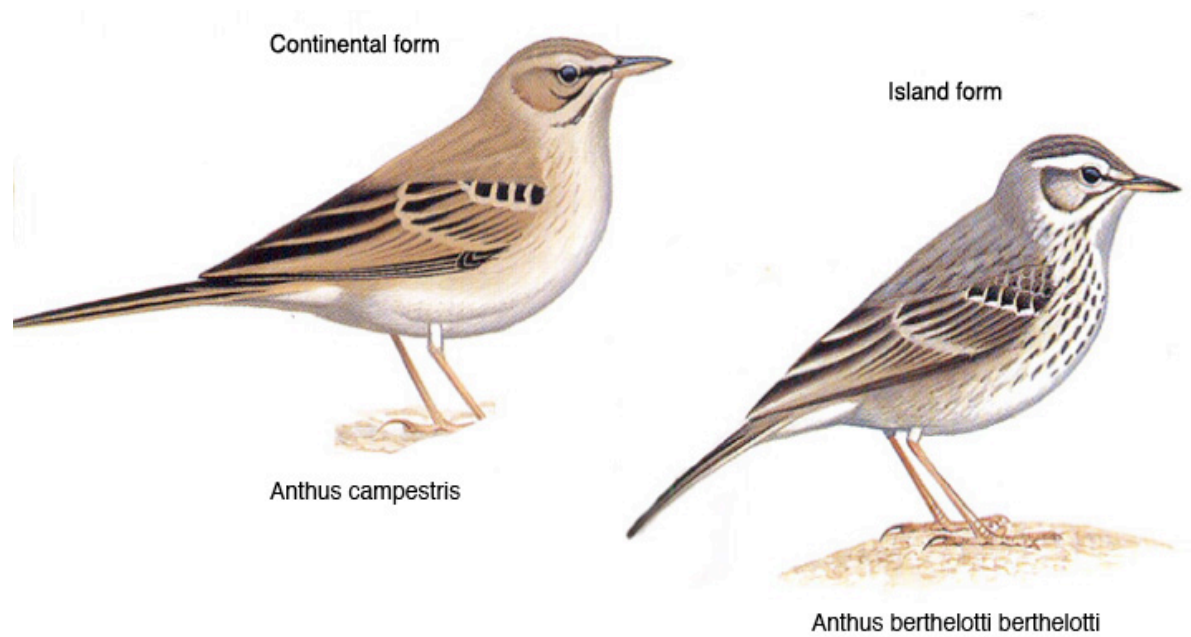


**Figure 4.2.** Scatter plot of the Log transformed initial mass of continental species vs their proportional change in mass in the island form.

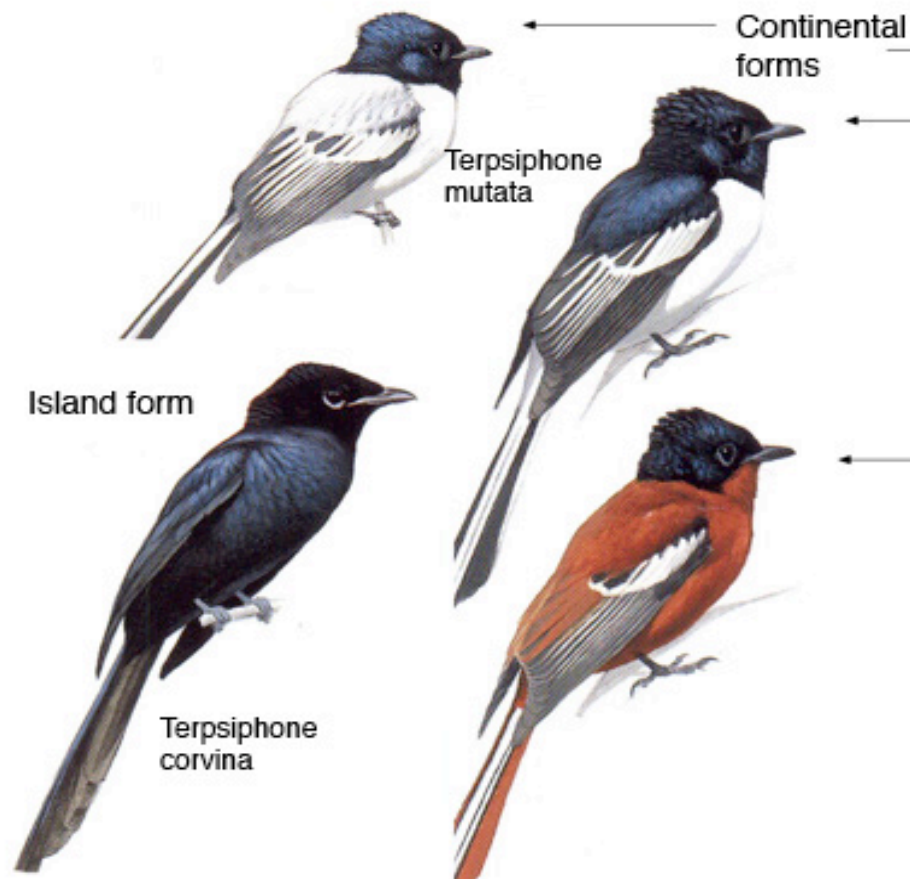




**Figure 4.3.** Example of a reduction in carotenoid pigmentation of an island species compared to its continental counterpart.



**Figure 4.4.** Example of an increase in melanin pigmentation in an island species compared to its continental counterpart. Note also the reduction in the buff wash (carotenoid pigments) in the island species as well.



**Figure 4.5.** Example of increased melanism in island species compared to their continental counterpart, resulting in reduction of contrasting patterns and loss of rufous forms. The result is a 'duller' island species.

## Chapter 5

### 5.1 General discussion

In the preceding chapters I was able to reveal some exciting differences between insular and continental passerines. Using New Zealand as a case study, I was able to test if some of these patterns were the result of evolutionary histories with different predator regimes. What became obvious though, was that the avifauna of New Zealand and other smaller more isolated islands is unique in a number of ways, including their life history, behaviour and morphology.

I initially suggested a ‘predation’ hypothesis as proposed by Briskie et al. (1999) to explain the differences in the nestling begging vocalisations of New Zealand and continental passerines. In fact, I found that the vocalisations of New Zealand and continental nestlings were similar in a number of ways, including decibel level and call length. However, New Zealand species did have broader frequency ranges, and interestingly, higher maximum frequencies than the continental species. The latter of these two observations is contrary to the predation hypothesis that assumes species under low predation pressure would utilise lower frequencies. Due to New Zealand species having only relatively low predation pressure (at least historically), one of the few remaining pressures shaping the evolution of begging calls is a metabolic cost. As I have elucidated to earlier, these costs are probably minimal (Chappell and Bachman 2002), but in some circumstances they could be important. Lower frequency calls are known to be more costly to produce than higher frequency calls (Roberts et al. 1983). Perhaps then New Zealand birds are using higher frequencies as

they are the least costly to produce energetically, and thereby reduce the effect of exaggerated begging displays on nestling growth and development. I also presented the possibility that the characteristics of the vocalisations that were most similar between the New Zealand and continental species were those that were plastic enough to have changed in the time since the introduction of mammalian predators. Since kiore (*Rattus exulans*) have been present in New Zealand for over 1000 years (Holdaway 1989), the native birds have had between 300 and 500 generations to evolve modifications to their vocalisations that would reduce conspicuousness to rodents. Although within such a timeframe morphological changes are unlikely, behavioural adaptations are very possible, and could be responsible for the similarities of some of the characteristics I measured in chapter 2.

My third chapter again tested a predation-related hypothesis for the modification of preen waxes during the incubation phase of the breeding cycle. Reneerkens et al. (2005) first proposed the ‘olfactory crypsis’ theory for the phenomenon he observed of incubating sandpipers (Family: Scolopacidae) shifting to produce less volatile, predominantly diester preen waxes, then reversing this shift to more volatile monoesters upon cessation of incubation and brooding. I found that the introduced continental species, which evolved alongside mammalian predators, showed a shift to producing less volatile preen waxes in the breeding season. The majority of New Zealand birds however, which have evolved in the absence of mammals, showed no shift in wax volatility in the breeding season. In the case of the South Island robin (*Petroica a. australis*) a shift to even more volatile preen waxes in the breeding season was observed. All of these findings appear to support the ‘olfactory crypsis’ theory for the shifting to less volatile preen waxes in the breeding season. What was also evident from my study was that New Zealand species, which

are not constrained by the small requirements for olfactory crypsis as continental species, may use preen waxes for functions other than those put forward by previous research. These functions included reducing feather wear, increasing water repellency, providing UV protection and/or possibly repelling lice, mites and other macro-ectoparasites (Elder 1954; Kolattukudy et al. 1987; Montalti and Salibian 2000; Sinninghe Damste et al. 2000; Sweeney et al. 2004). There is the possibility that with their isolation on a predator free island, New Zealand birds have adopted additional functions for their preen waxes. Unconstrained by a requirement for olfactory crypsis, New Zealand species could be producing waxes that are adapted purely for optimal ecophysiological function, with a by-product of this functionality being increased volatility. Or perhaps the incorporation of an additional function of communication has resulted from the years of mammal free isolation. Perhaps the South Island robin, which produced significantly more volatile preen waxes during the breeding season than in the non-breeding season, is using increased volatility of its waxes in the breeding season as a “perfume” in mate choice. It is clearly an area worth investigating further.

Chapter 4 looked at the evolutionary patterns of insular passerines compared to their continental counterparts. Specifically I looked at changes in body and bill size, carotenoid pigmentation, melanin pigmentation and the overall ‘dullness’ of island species compared to their continental counterparts. I found that island species were significantly larger, had larger bills and were significantly ‘duller’ than their continental counterparts. This dullness can be attributed to reductions in carotenoids and a significant increase in the melanin pigmentation of island species. These findings align with previous observations of small species becoming larger on islands (van Valen 1965; Lomolino 1985). The passerines used in my study are small relative

to all bird species, and this is most possibly why I observed an overall increase in size, and not a dichotomous pattern of size change, as predicted by van Valen's (1973) 'island rule'. The 'island rule' predicts large species to decrease in size on islands, and small species to increase in size. I suggest that the species used in my analysis represent only those species that are predicted to increase in size. As such it does not disprove the island rule.

The changes in colour observed in my analysis are possibly due to founder effects (Mayr 1942), or perhaps due to a change in the dynamic of sexual and natural selection (Doucet et al. 2004). The founder effects model (Mayr 1942) argues that evolution is faster in small populations, like those of small isolated islands. This is due to the inbreeding of insular populations perpetuating increased homozygosity, decreases in genotypic and phenotypic variability and increased gene loss, all of which result in rapid divergent evolution of island species. This presents a mechanism for the speed and repeated occurrence of non-specific evolutionary change in island species, but fails to present a reason for the repeated incidence of similar changes (i.e. increased size, increased melanins) in island species. Another possibility is that island females are no longer selecting males on the basis of 'continental' cues of quality based on plumage brightness. This is because the island environment, with its reduced intra and interspecific competition, presents a new paradigm on which to base a judgment of quality. Therefore females may no longer recognise colourful males as being 'fit' males as the honesty of the signal is compromised. This can be the result of reduced competition for resources, which may be precursors for expression of the signal, or simply reduced competition due to a sex bias, or increased territory availability. Making a distinction as to what exactly is responsible for the patterns I have observed is beyond the scope of this study. However, integrating

theories like those above may hold the key for discovering precisely how and why patterns of increased size and dullness are regularly exhibited by island species.

Together my 3 studies paint a complex, and interesting, pattern of changes that occur in island birds. A loss in carotenoid pigmentation, contrasting plumage and other conspicuous and generally sexually selected traits may indicate that island species are using different cues in place of visual ones as mechanisms of mate choice. For example, the South Island robin is far duller than its trans-Tasman cousins, aptly named the Flame robin (*Petroica phoenicea*), Rose robin (*P. rosea*), Red-capped robin (*P. goodenovii*) and Scarlet robin (*P. multicolor*). With the loss of carotenoid pigmentation upon isolation in New Zealand one can imagine that the South Island robin adopted alternative means of signalling quality or compatibility to potential mates. Perhaps South Island robins produce more volatile preen waxes in the breeding season as a chemosensory “colour”. Such a change might be expected in an island system where no mammalian predators may constrain the evolution of chemosensory cues from the preen waxes.

Finally, my study suggests a number of further investigations on the insular evolution of birds. For example, a number of additional studies could be carried out to flesh out the model of how island environments affect the evolution of avian species. Specifically the analysis of adult song on island groups and how this compares to their continental counterparts may provide more information regarding the changes in sexual selection on islands, and what medium island birds use to convey their signals of quality. An expansion of my analyses to include the preen waxes of birds from other island groups is also needed and would allow differentiation between the discrete effects of New Zealand on evolution of preen waxes and the effects of isolation on islands as a whole.



Different traits have varying degrees of plasticity, and the timeframe by which a trait can undergo functional adaptations can be very important. This can be crudely assessed by looking at mainland populations of native New Zealand species which have been under mammalian predation for around 1000 years (Holdaway 1989), and comparing them to populations of the corresponding species on offshore islands that have never been colonised by mammals. One could look at differences in begging vocalisations, colouration, adult song, preen waxes and a whole host of other traits and devise some sort of spectrum of plasticity, assessing on temporal scales the amount of change exhibited in each trait. This would be important in the breaking down of behavioural and physiological adaptation on islands; thus allowing one to distinguish which traits are a result of the evolutionary history of islands, and which traits have succumbed to pressures of continental introductions and anthropogenic modifications, and have undergone compensatory adaptations.

Finally, a thorough understanding of how island birds have changed and why, will provide critical insight into what makes such species so vulnerable to the introduction of exotic mammalian predators. It is only by fully understanding the evolutionary history of island birds, that we can then devise the best means to ensure their protection and prevent their future extinction.

## 5.2 References.

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## Appendix 1.

Pair #	Species	Region
1	<i>Pachyramphus aglaiae albiventris</i>	West Mexico
	<i>Pachyramphus aglaiae insularis</i>	Tres Marias Island
2	<i>Myiopagis viridicata jaliscensis</i>	West Mexico
	<i>Myiopagis viridicata minima</i>	Tres Marias Island
3	<i>Elaenia spectabilis</i>	South America
	<i>Elaenia ridleyana</i>	Fernando de Noronha
4	<i>Phaeomyias murina</i>	Central and South America
	<i>Nesotriccus ridgwayi</i>	Coco Island
5	<i>Pyrocephalus rubinus piurae</i>	North and South America
	<i>Pyrocephalus rubinus nanus</i>	Galapagos Islands
6	<i>Muscisaxicola maclovianus mentalis</i>	Southern South America
	<i>Muscisaxicola m. maclovianus</i>	Falkland Islands
7	<i>Myiarchus tyrannulus</i>	North and South America
	<i>Myiarchus magnirostris</i>	Galapagos Island
8	<i>Cincloides cincloides oustaleti</i>	Chile
	<i>Cincloides oustaleti baeckstroemii</i>	Juan Fernandez Islands
9	<i>Aphrastura spinicauda</i>	Chile
	<i>Aphrastura masafuerae</i>	Juan Fernandez Islands
10	<i>Anthornis melanura melanura</i>	New Zealand
	<i>Anthornis melanura melanocephala</i>	Chatham Islands
11	<i>Prothemadera n. novaeseelandiae</i>	New Zealand
	<i>Prothemadera novaeseelandiae chathamensis</i>	Chatham Islands

12	<i>Gerygone i. igata</i>	New Zealand
	<i>Gerygone igata modesta</i>	Norfolk island
13	<i>Gerygone i. igata</i>	New Zealand
	<i>Gerygone albofrontata</i>	Chatham Islands
14	<i>Strepera g. graculina</i>	Australia
	<i>Strepera graculina crissalis</i>	Lord Howe Island
15	<i>Coracina c. cinerea</i>	Madagascar
	<i>Coracina typical</i>	Mauritius Island
16	<i>Pachycephala p. pectoralis</i>	Australia
	<i>Pachycephala pectoralis xanthoprocta</i>	Norfolk Island
17	<i>Vireo g. griseus</i>	North America
	<i>Vireo griseus bermudianus</i>	Bermuda Islands
18	<i>Vireo h. hypochryseus</i>	West Mexico
	<i>Vireo hypochryseus sordidus</i>	Tres Marias Islands
19	<i>Vireo olivaceus</i>	North and South America
	<i>Vireo gracilirostris</i>	Fernando de Noronha Island
20	<i>Rhipidura albiscapa alisteri</i>	Australia
	<i>Rhipidura albiscapa pelzelni</i>	Norfolk Island
21	<i>Rhipidura f. fuliginosa</i>	New Zealand (South Island)
	<i>Rhipidura fuliginosa penitus</i>	Chatham Islands
22	<i>Terpsiphone m. mutata</i>	Madagascar
	<i>Terpsiphone corvina</i>	Seychelles Islands
23	<i>Corvus corax tingitanus</i>	North Africa
	<i>Corvus corax canariensis</i>	Canary Islands

24	<i>Petroica b. boodang</i>	Eastern Australia
	<i>Petroica m. multicolour</i>	Norfolk Island
25	<i>Petroica m. macrocephala</i>	New Zealand (South Island)
	<i>Petroica macrocephala chathamensis</i>	Chatham Islands
26	<i>Petroica australis</i>	New Zealand
	<i>Petroica traversi</i>	Chatham Islands
27	<i>Parus caeruleus ultramarinus</i>	North West Africa
	<i>Parus caeruleus teneriffae</i>	Canary Islands
28	<i>Phedina borbonica</i> <i>madagascariensis</i>	Madagascar
	<i>Phedina b. borbonica</i>	Mauritius, Reunion
29	<i>Progne murphyi</i>	Peru/Chile
	<i>Progne modesta</i>	Galapagos Islands
30	<i>Alaemon a. alaudipes</i>	North Africa
	<i>Alaemon alaudipes boavistae</i>	Cape Verde Islands
31	<i>Ammomanes cinctura arenicolor</i>	North Africa
	<i>Ammomanes c. cinctura</i>	Cape Verde Islands
32	<i>Calandrella rufescens apetzii</i>	Southern Iberia
	<i>Calandrella rufescens polatzeki</i>	Canary Islands
33	<i>Alauda arvensis harterti</i>	North West Africa
	<i>Alauda razae</i>	Cape Verde Islands
34	<i>Eremopterix nigriceps albifrons</i>	Africa
	<i>Eremopterix n. nigriceps</i>	Cape Verde Islands
35	<i>Hypsipetes m. madagascariensis</i>	Madagascar
	<i>Hypsipetes crassirostris</i>	Seychelles

36	<i>Megalurus p. punctatus</i>	New Zealand
	<i>Megalurus punctatus rufescens</i>	Chatham Islands
37	<i>Nesillas t. typical</i>	Madagascar
	<i>Nesillas aldabrana</i>	Aldabra Islands
38	<i>Acrocephalus rufescens senegalensis</i>	Senagal/West Africa
	<i>Acrocephalus brevipennis</i>	Cape Verde Islands
39	<i>Acrocephalus newtoni</i>	Madagascar
	<i>Acrocephalus sechellensis</i>	Seychelles Islands
40	<i>Phylloscopus c. collybita</i>	Europe
	<i>Phylloscopus c. canariensis</i>	Canary Islands
41	<i>Sylvia atricapilla heineken</i>	North West Africa
	<i>Sylvia atricapilla gularis</i>	Cape Verde and Azores Islands
42	<i>Sylvia c. conspicillata</i>	Europe and Africa
	<i>Sylvia conspicillata orbitalis</i>	Madiera, Canary and Cape Verde Islands
43	<i>Zosterops l. lateralis</i>	Tasmania
	<i>Zosterops lateralis tephropleurus</i>	Lord Howe Island
44	<i>Zosterops l. lateralis</i>	Tasmania
	<i>Zosterops t. tenuirostris</i>	Norfolk Island
45	<i>Zosterops m. maderaspatanus</i>	Madagascar
	<i>Zosterops maderaspatanus aldabransis</i>	Aldabra Island
46	<i>Regulus i. ignicapilla</i>	Western Europe
	<i>Regulus ignicapilla madeirensis</i>	Madeira
47	<i>Regulus r. regulus</i>	Europe
	<i>Regulus regulus azoricus</i>	Azores Island



48	<i>Regulus r. regulus</i>	Europe
	<i>Regulus regulus teneriffae</i>	Western Canary Islands
49	<i>Regulus calendula grinnelli</i>	Western U.S.A
	<i>Regulus calendula obscurus</i>	Guadelupe Island
50	<i>Salpinctes o. obsoletus</i>	Mexico
	<i>Salpinctes obsoletus guadeloupensis</i>	Guadelupe Island
51	<i>Cistothorus platensis hornensis</i>	Southern South America
	<i>Cistothorus platensis falklandicus</i>	Falkland Islands
52	<i>Thryomanes bewickii charienturus</i>	North Baja California
	<i>Thryomanes bewickii brevicaudus</i>	Guadelupe Island
53	<i>Troglodytes aedon</i>	Western U.S.A
	<i>Troglodytes sissonii</i>	Socorro Island
54	<i>Thryomanes felix pallidus</i>	Western Mexico
	<i>Thryomanes felix lawrencii</i>	Tres Marias Islands
55	<i>Troglodytes aedon</i>	Mexico
	<i>Troglodytes tanneri</i>	Clarion Island
56	<i>Troglodytes musculus chilensis</i>	Southern South America
	<i>Troglodytes cobbi</i>	Falkland Islands
57	<i>Melanotis c. caeruleascens</i>	Mexico
	<i>Melanotis caeruleascens longirostris</i>	Tres Marias Islands
58	<i>Aplonis m. metallica</i>	North East Australia
	<i>Aplonis f. fusca</i>	Norfolk Island
59	<i>Sturnus v. vulgaris</i>	Europe
	<i>Sturnus vulgaris granti</i>	Azores Islands

60	<i>Sialia s. sialis</i>	Eastern U.S.A
	<i>Sialia sialis bermudensis</i>	Bermuda Island
61	<i>Myadestes o. occidentalis</i>	North West Mexico
	<i>Myadestes occidentalis insularis</i>	Tres Marias Islands
62	<i>Turdus pelios</i>	Africa
	<i>Turdus o. olivaceofuscus</i>	Sao Tome Island
63	<i>Turdus m. merula</i>	Europe
	<i>Turdus merula cabreræ</i>	Madeira and Canary Islands
64	<i>Turdus falcklandii magellanicus</i>	Southern South America
	<i>Turdus f. falcklandii</i>	Falkland Islands
65	<i>Turdus r. rufopalliatus</i>	Southern Mexico
	<i>Turdus rufopalliatus graysoni</i>	Tres Marias Islands
66	<i>Erithacus r. rubecula</i>	Europe
	<i>Erithacus rubecula superbus</i>	Tenerife and Grand Canaria Islands
67	<i>Copsychus saularis</i>	India
	<i>Copsychus sechellarum</i>	Seychelles Islands
68	<i>Saxicola torquatus rubicola</i>	Europe and North West Africa
	<i>Saxicola d. dacotinae</i>	Canary Islands
69	<i>Saxicola torquatus sibilla</i>	Madagascar
	<i>Saxicola torquatus tectes</i>	Reunion Island
70	<i>Anabathmis reichenbachii</i>	Africa
	<i>Anabathmis hartlaubii</i>	Principe Island
71	<i>Cinnyris s. souimanga</i>	Madagascar
	<i>Cinnyris souimanga abbotti</i>	Aldabra Island

72	<i>Cinnyris s. souimanga</i>	Madagascar
	<i>Cinnyris dussumieri</i>	Seychelles Islands
73	<i>Passer motitensis</i>	Africa
	<i>Passer iagoensis</i>	Cape Verde Islands
74	<i>Ploceus v. vitellinus</i>	Africa
	<i>Ploceus vitellinus peixotoi</i>	Sao Tome Islands
75	<i>Foudia madagascariensis</i>	Madagascar
	<i>Foudia sechellarum</i>	Seychelles Islands
76	<i>Motacilla c. cinerea</i>	Europe
	<i>Motacilla cinerea patriciae</i>	Azores Island
77	<i>Anthus n. novaeseelandiae</i>	New Zealand (South Island)
	<i>Anthus novaeseelandiae chathamensis</i>	Chatham Islands
78	<i>Anthus campestris</i>	Europe
	<i>Anthus b. berthelotti</i>	Canary Islands
79	<i>Anthus correndera chilensis</i>	Southern South America
	<i>Anthus correndera grayi</i>	Falkland Islands
80	<i>Anthus correndera chilensis</i>	Southern South America
	<i>Anthus antarcticus</i>	South Georgia Island
81	<i>Fringilla coelebs balearica</i>	South West Europe
	<i>Fringilla coelebs canariensis</i>	Canary Islands
82	<i>Fringilla coelebs africana</i>	Europe
	<i>Fringilla t. teydea</i>	Tenerife Island
83	<i>Serinus serinus</i>	Europe
	<i>Serinus canaria</i>	Canary Islands

84	<i>Carduelis p. psaltria</i>	North Mexico
	<i>Carduelis psaltria witti</i>	Tres Marias Islands
85	<i>Carduelis cannabina mediterranea</i>	Iberia
	<i>Carduelis cannabina harterti</i>	Canary Islands
86	<i>Bucanetes githagineus zedlitzi</i>	Europe and Africa
	<i>Bucanetes githagineus amentum</i>	Canary Islands
87	<i>Carpodacus mexicanus ruberrimus</i>	North West Mexico
	<i>Carpodacus mexicanus amplus</i>	Guadeloupe Island
88	<i>Pyrrhula pyrrhula iberiae</i>	South West Europe
	<i>Pyrrhula pyrrhula murina</i>	Azores Islands
89	<i>Parula pitiayumi pulchra</i>	North West Mexico
	<i>Parula pitiayumi insularis</i>	Tres Marias Island
90	<i>Dendroica petechia peruviana</i>	South America
	<i>Dendroica petechia aurealo</i>	Galapagos Islands
91	<i>Granatellus v. venustrus</i>	West Mexico
	<i>Granatellus venustus francescae</i>	Tres Marias Islands
92	<i>Icterus p. pustulatus</i>	Mexico
	<i>Icterus pustulatus graysoni</i>	Tres Marias Islands
93	<i>Sturnella l. loyca</i>	Southern South America
	<i>Sturnella loyca falklandica</i>	Falkland Islands
94	<i>Junco hyemalis mearnsi</i>	Western North America
	<i>Junco hyemalis insularis</i>	Guadalupe Island
95	<i>Melanodera melanodera</i>	Southern South America
	<i>princetoniana</i>	
	<i>Melanodera m. melanodera</i>	Falkland Islands

96	<i>Pipilo maculatus</i>	Mexico
	<i>Pipilo maculatus socorroensis</i>	Socorro Island
97	<i>Piranga b.bidentata</i>	Western Mexico
	<i>Piranga bidentata flammea</i>	Tres Marias Island
98	<i>Cardinalis cardinalis sinaloensis</i>	Southern and Western Mexico
	<i>Cardinalis cardinalis mariae</i>	Tres Marias Island